Monitoring Report for Bacterial Source Tracking

Segments 0806, 0841, and 0805 of the Trinity River

Bacteria TMDL

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Introduction

1.1 Background and Water Quality Standards

Protection of our state's water resources is one of the most significant environmental challenges with which Texans currently contend. Texas' water resources are being depleted or impacted daily, through actions such as overuse, urban development, agricultural activities, and wetland degradation. Conservation and protection of our water resources will be one of the most important measures undertaken by local, state and federal agencies, as well as environmental groups, during the 21st century. As our states population increases, so does the need for dependable and employable water resources. Large cities such as Houston, San Antonio, and the Dallas/Fort Worth metroplex will require the largest percentages of clean, dependable water. In 2000 the estimated population of the Texas Water Development Board's Region C, which includes the Dallas /Fort Worth metroplex, was 5.3 million. This represents almost 25 percent of the states total population. Over 90 percent of that population is found within the Dallas/ Fort Worth area, and 95 percent were estimated to live in the Trinity River basin. The region's population is expected to practically double by 2050, increasing the water supply requirements of the region, as well (TRA, 2003). This increasing need emphasizes that currently reliable water sources be protected and those that display negative impacts be restored and then maintained.

Water quality standards were developed in order to ensure that designated uses for water bodies are met (TNRCC, 2000). These standards include specific criteria set forth by the Texas Commission on Environmental Quality (TCEQ) in the Texas Surface Water Quality Standards (TSWQS) (Title 30 Texas Administrative Code (TAC) Chapter 307). Section 303(d) of the Clean Water Act (CWA) and the U.S. Environmental Protection Agency (USEPA) Water Quality Planning and Management Regulations (40 Code of Federal Regulations [CFR] Part 130) require that states perform total maximum daily loads (TMDLs) for water bodies not meeting water quality standards. TMDLs establish the allowable loadings of pollutants for a water body based on the relationship between pollution sources and in-stream water quality conditions. TMDLs allow each state to implement water-quality based controls to reduce pollution from both point and nonpoint sources and to restore and maintain the quality of its water resources (USEPA, 1991).

Designated uses for water bodies in Texas include: aquatic life use, public water supply use, fish consumption use, oyster waters use, non-contact recreation use, and contact recreation use (TCEQ, 2003a). For the purposes of this report, *contact recreation use* is the only use that will be addressed. *Contact recreation use* is a use that is assigned to all water bodies in Texas, except in special situations (e.g., where ship or barge traffic make contact recreation use unsafe, thus requiring the designation of *non-contact recreation use)* (TCEQ, 2003a).

Contact recreation use is based upon the concentration of fecal coliform bacteria identified in a particular body of water. Fecal coliforms are gram negative, facultative anaerobic, lactose-fermenting bacteria that are commonly found in the intestines of homeotherms (Talaro and Talaro, 1999). *E. coli*, a species of coliform bacteria, is often used as an indicator of the

possible presence of fecal pathogens in water, because its concentration in water is relatively easy to measure and it is often the most abundant species of the fecal coliform bacteria (Talaro and Talaro, 1999). Applicable State of Texas water quality criteria for contact recreation use in freshwater state that the geometric mean concentration for *E. coli* should not exceed 126 colony forming units (cfu) per 100 ml and the single sample concentration should not exceed 394 (cfu) per 100 ml in greater than 25% of the individual samples. Until recent years, TCEQ has considered that a water body is fully supporting if 25% or less of the sample sets are in exceedance and not supporting if greater than 25% of the sample sets are in exceedance. However, TCEQ recognizes that the chance of falsely classifying a station or assessment unit as impaired (Type I Error) is relatively high for the historically utilized method. Therefore, new exceedance criteria was developed by TCEQ, the binomial method, in order to maintain a Type I error probability below 20%. (TCEQ, 2003a).

1.2 Bacterial Source Tracking

E. coli is a common inhabitant of animal and human intestines, and a few strains, notably strain O157:H7, are pathogenic (Talaro and Talaro, 1999). Recent studies have shown that *E. coli* isolates from humans and various other host animals (e.g., cattle, chickens, and pigs) may differ both genetically and phenotypically. These differences allow the use of genetic and biochemical tests in order to identify the original host animal, a process often referred to as bacterial source tracking (BST).

BST is based upon two principles. The first principle is that the bacterial population genetic structure is clonal. This principle is a well-established element of microbial genetics. Bacteria reproduce by binary fission or dividing in half. The two daughter cells that are generated as a result of this cell division are virtually identical in all aspects. All descendents of a common ancestral cell are genetically related to each other. Over time, members of a given clone may accumulate genetic changes, which will cause them to diverge from the main lineage and to form one or several new clonal groups. BST makes use of the clonal population structure of bacteria to classify organisms based on their genetic fingerprints into groups of clonal descent.

The second principle behind the BST methodology is the assumption that within a given species of bacteria, various members have adapted to living/environmental conditions in specific hosts/environments. As a result, there is a high degree of host specificity among bacterial strains that are seen in the environment. A bacterial strain that has adapted to a particular environment or host (e.g., animal intestinal tract) is capable of colonizing that environment and competing favorably with members of the host's indigenous flora. Such a bacterial strain is called a resident strain. Resident strains are usually shed from their host over a long period of time, thus providing a reliable, characteristic signature of their source. A transient strain is a bacterial strain that is introduced into a new environment or host but cannot colonize and persist in that environment. If a host is sampled over time for a given species of bacteria, a few resident strains are consistently being shed while a large number of transient strains are shed for brief lengths of time. Many transient strains have also been found to be non-specific with regard to the host species. Hartl and Dykhuizen (1984) illustrate this point. Over a period of 11 months, 22 fecal samples were taken from a single individual. A total of 550 *E. coli* isolates were characterized, of which two were considered to be resident strains, appearing 252 times. Data show that by

using this subtyping method (ribosomal RNA typing using two restriction enzyme reactions) more than 96% of *E. coli* strains are seen in only one host species (or group of related species) (Mazengia, 1998). Thus, it appears that only about 4% of the *E. coli* strains are transient and not attributable to one specific source.

Historically, the key methodological problem in tracing sources of microbial contamination in the environment was the lack of a universal single-reagent typing scheme for bacteria. This problem has been overcome by the work of several investigators in the fields of population genetics, molecular systematics, and molecular epidemiology. In 1986 Grimont and Grimont showed that DNA probes corresponding to specific regions of the ribosomal ribonucleic acid (rRNA) operon could be used to differentiate bacteria. Stull et al. (1988) and LiPuma et al. (1988) used the rRNA operon to study the molecular epidemiology of several species of bacteria. In order to trace the indicator bacterium, *E. coli*, from the water to its specific source, the bacterial strain must first be uniquely identified. Populations of *E. coli*, like other bacteria, are composed essentially of a mixture of strains of clonal descent. Due to the relatively low rates of recombination, these clones remain more or less independent (Selander et al., 1987). These clones, or strains of bacteria, are uniquely adapted to their own specific environments. As a result the *E. coli* strain that inhabits the intestines of one species is genetically different from the strain that might inhabit another. Ribosomal ribonucleic acids, which are integral to the machinery of all living cells and tend to be very highly conserved, make an ideal choice of target for interstrain differentiation. Since the *E. coli* chromosome contains seven copies of the rRNA operon, an rDNA probe can be used as a definitive taxonomic tool (Grimont and Grimont, 1986). That is, when digested with restriction enzymes, resolved by agarose gel electrophoresis, transferred to a membrane and hybridized with an rRNA probe, an *E. coli* chromosome will produce several bands to create a specific restriction fragment length polymorphism (RFLP) pattern that can be used to uniquely identify the bacterial strain.

Molecular tools appear to hold the greatest promise for BST, providing the most conclusive characterization and the highest level of discrimination for isolates. Of the molecular tools available, ribosomal ribonucleic acid genetic fingerprinting (Ribotyping) and pulsed-field gel electrophoresis (PFGE) are emerging as versatile and feasible BST techniques. However, reference "libraries" of bacterial genetic fingerprints and antibiotic resistance profiles are needed to correctly identify the source of bacteria isolated from environmental water samples. PFGE, while excellent at resolving different source species, requires a very large and expensive library due to the high variation in PFGE profiles. A phenotypic characterization method, antibiotic resistance analysis, also has the potential to identify the human or animal origin of isolates. However, there is substantial uncertainty over the efficacy of antibiotic resistance analysis at distinguishing bacterial sources. Ribotyping is a common bacterial source tracking method because it balances high source specificity with moderate requirements for library size.

The pattern of DNA fragments corresponding to the rRNA operon is referred to as the ribotype. Ribotyping has been useful in many studies to differentiate between bacterial strains that would have otherwise been difficult or impossible to distinguish. Fisher et al. (1993) followed the transmission of *Pseudomonas cepia* from environmental sources to and between cystic fibrosis patients and discovered the majority of cases contracted cystic fibrosis from one of two treatment centers. Moyer et al. (1992) used ribosomal RNA typing to identify the

Aeromonas spp. strains responsible for several waterborne gastroenteritis episodes in a community and was able to trace the contamination to specific locations in water treatment and distribution systems. Barloga and Harlander (1991) compared several typing methods for distinguishing between strains of *Listeria moncytogenes* implicated in a food-borne illness and found that ribotyping was the preferred method due to its precision and reproducibility. Atlas et al. (1992) described the technology of ribotyping as applicable to the tracking of genetically engineered microorganisms (GEMs) in the environment.

1.3 Review of Trinity River Historical Data

Recent environmental monitoring along the West Fork Trinity River (Segments 0806 and 0841) and the Upper Trinity River (Segment 0805) has shown that *E. coli* bacteria have reached unacceptably high concentrations, exceeding the specified criteria for contact recreation use in portions of those segments (Figure 1-1). Descriptions of those segments, beginning at the uppermost portion of the watershed, and their impaired reaches are listed as follows:

Segment 0806 (West Fork Trinity River below Lake Worth), a 33 mile freshwater stream, runs from Lake Worth in Tarrant County to a point immediately upstream of the confluence with Village Creek, also in Tarrant County. *E. coli* impairments have been reported for the lower 22 miles of the segment (Texas 303(d) list; 2002, 2004 draft).

Segment 0841 (Lower West Fork Trinity River), a 27 mile freshwater stream, begins immediately upstream of the confluence of Village Creek (where Segment 0806 ends) in Tarrant County and runs to a point immediately upstream of the confluence with the Elm Fork of the Trinity in Dallas County. *E. coli* impairments have been reported for the lower 14 miles of the segment (Texas 303(d) list; 2002, 2004 draft).

Segment 0805 (Upper Trinity River), a 100 mile freshwater stream, begins immediately upstream of the confluence with the Elm Fork (where Segment 0841 ends) in Dallas County and runs to a point immediately upstream of the confluence with Cedar Creek Reservoir's discharge canal, along the Henderson/ Navarro County line. *E. coli* impairments have been reported for:

- the upper 8 miles of the segment.,
- the 11-mile reach near South Loop 12., and
- the 25-mile reach near SH 34 (Texas 303(d) list; 2002, 2004 draft).

In order to properly assess the condition of the stream, an investigation of historical water quality data, in addition to the recent sampling, needed to be conducted. Data sources included the TCEQ Texas Regulatory Activity and Compliance System (TRACS) database and the Trinity River Authority for recent data not yet included in TRACS. Available *E. coli* data from September 2000 to August 2005 were reviewed for the affected segments. These historical data were used to assess the water quality according to TSWQS (TCEQ, 2003b) procedures. TSWQS (2003) states that the most recent five years of data should be used in order to properly assess the status of the stream. The historical data indicated that 13 monitoring stations had historical *E. coli* data during this five-year time period. According to the historical data, eight of the stations failed to meet the contact recreation use (Figure 1-2; Table 1-1)—stations 17863, 11080, 16120,

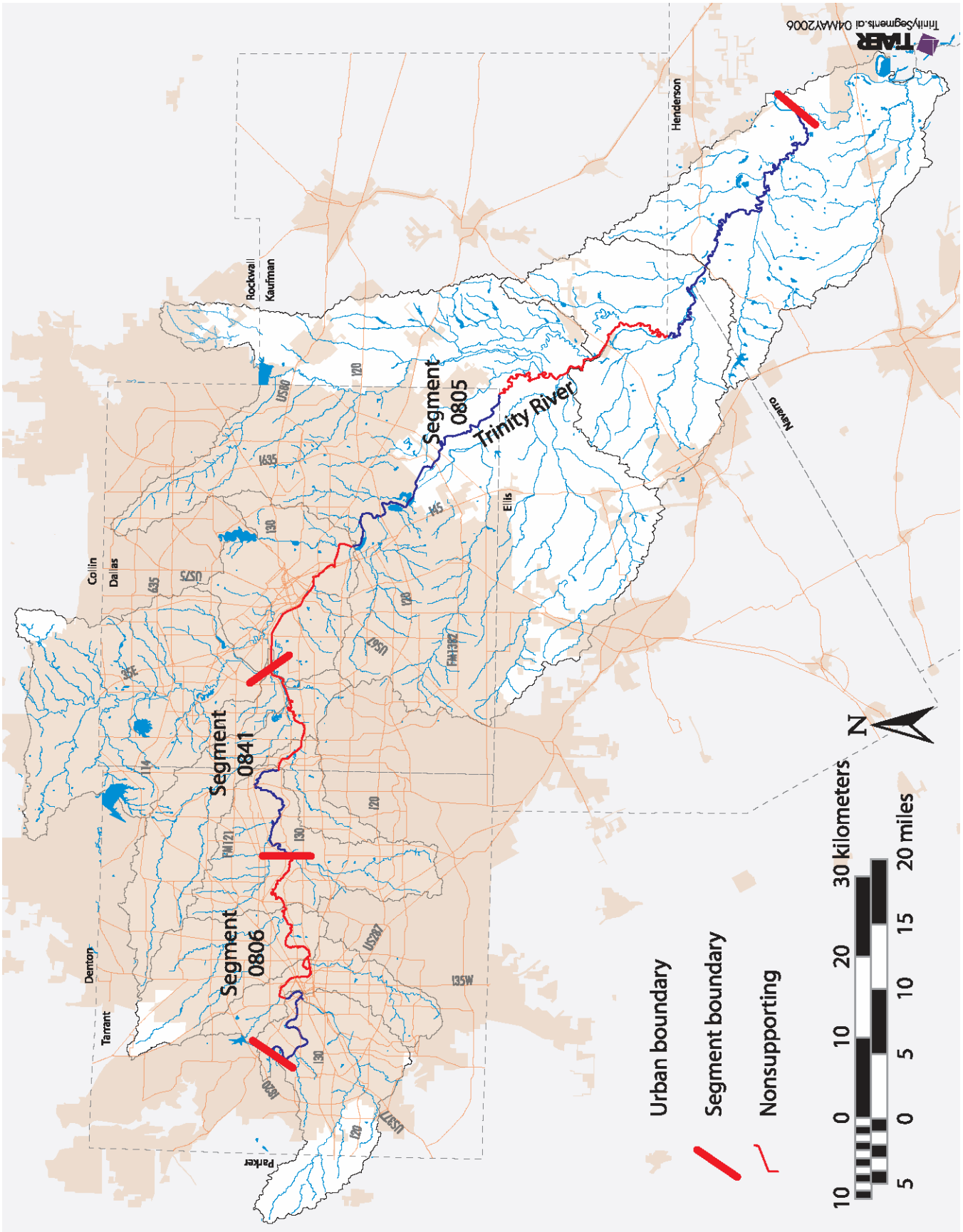

Figure 1-1. Trinity River study area showing nonsupporting reaches.

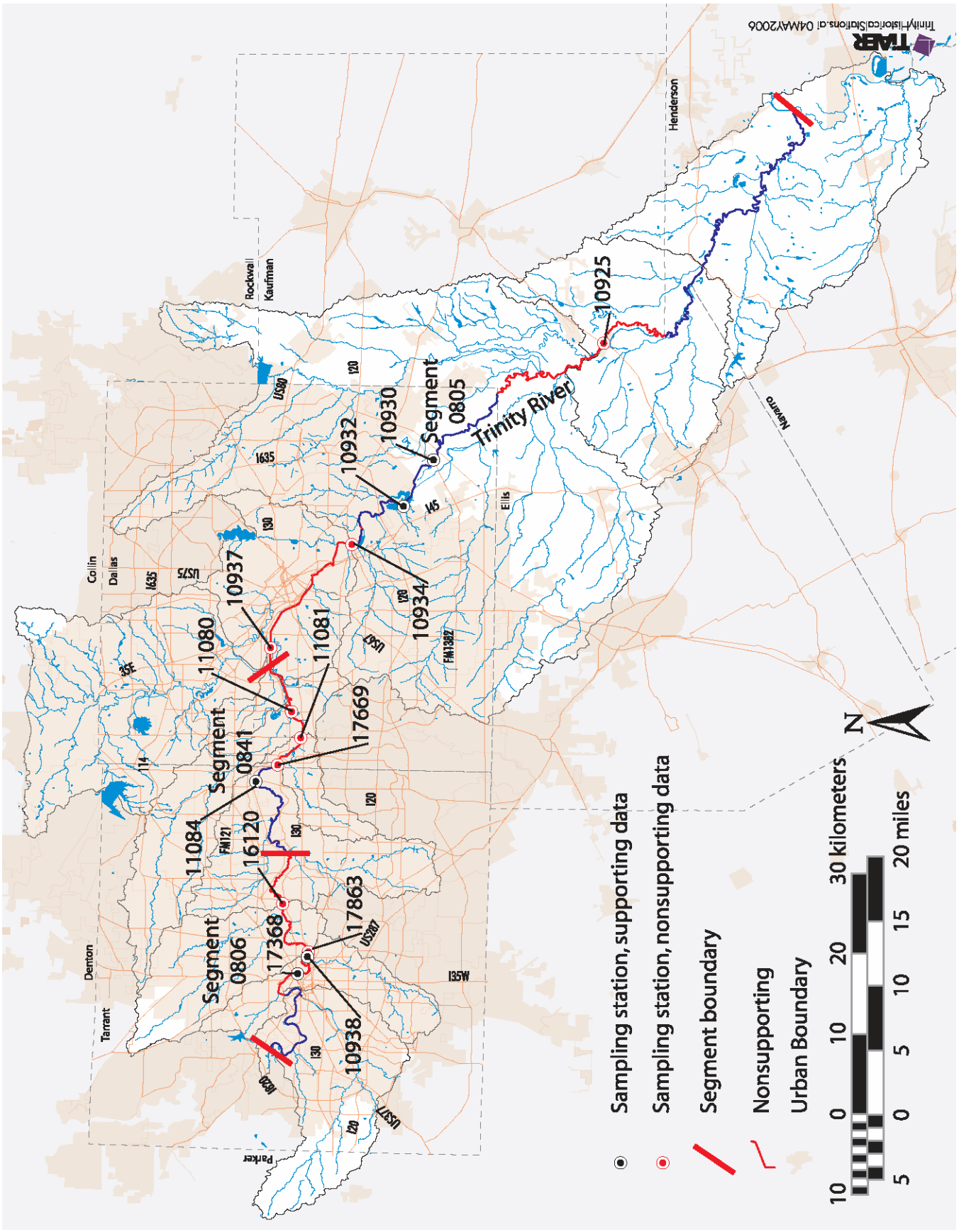

Figure 1-2. Trinity River study area showing stations with *E. coli* data used to assess contact recreation use.

Table 1-1. Summary of historical *E. coli* data for August 2000 through September 2005, including support and nonsupport designations. Shaded stations indicate nonsupport of contact recreation use due to single sample exceedances and/or geometric mean exceedance.

Segment	Station ID	No. of Samples Aug. 2000-Sept. 2005	Min. Measured *E. coli* Conc.	Max. Measured *E. coli* Conc.	Single Sample Exceedences	Single Sample Percent Exceedances	Geometric Mean	Location
0806								
	17368	70	1	3640	12	17%	47	4th St./ Tarrant Co.
	10938	78	1	4840	17	22%	59	Beach St./ Tarrant Co.
	17863	31	2	24200	8	26%	131	Gateway Park/ Tarrant Co.
	16120	62	1	98040	23	37%	169	Handley-Ederville/ Tarrant Co.
0841								
	11084	16	18	452	1	6%	55	SH 360/ Tarrant Co.
	17669	38	17	4838	9	24%	159	Roy Orr/ Dallas Co.
	11081	38	2	19900	16	42%	278	Belt Line Rd./ Dallas Co.
	11080	33	13	3470	8	24%	170	South MacArthur Blvd./ Dallas Co.
0805								
	10937	46	12	24200	18	39%	238	Mockingbird Ln./ Dallas Co.
	10934	45	21	39700	19	42%	383	South Loop 12/ Dallas Co.
	10932	13	11	980	2	15%	85	Dowdy Ferry Rd./ Dallas Co.
	10930	35	3	1540	6	17%	64	Belt Line Rd. near Wilmer/ Dallas Co.
	10925	54	2	4840	17	31%	139	SH 34/ Ellis-Kaufman Co. Line
Fully Supporting Contact Recreation								
Not Supporting Contact Recreation								

17669, 11081, 0937, 10934, and 10925. Stations 17863 and 16120 are located in Segment 0806; stations 11080, 17669, and 11081 are located in Segment 0841; and stations 10937, 10934, and 10925 are located in Segment 0805. The geometric mean prevented all of these stations from meeting their contact recreation use. In addition, stations 16120, 11081, 10937, 10934, and 10925 also failed to meet the percent single sample exceedance criteria based on the binomial method.

1.4 Study Objectives

TCEQ's assessment of ambient bacteria data and more recent data assessed for the present study led to the conclusion that portions of Segments 0806, 0841, and 0805 do not support the contact recreation use. Consequently, TCEQ is using the Texas Institute for Applied Environmental Research (TIAER) at Tarleton State University as the lead performing entity to: (1) acquire data and information necessary to support modeling and assessment activities, (2) perform bacteria source tracking activities to support allocations of loadings, and (3) assist the TCEQ in preparing a TMDL. As the lead performing entity, TIAER organized a Project Team that included Parsons Water & Infrastructure, Inc. and James Miertschin & Associates, Inc., who both assisted with field efforts and data collection, and the Institute for Environmental Health, Inc. (IEH), who performed laboratory ribotyping of both ambient water and known source, library *E. coli* samples. This report addresses item (2) on bacteria source tracking. This report includes information on study area, characteristics, materials and methods of bacterial source tracking, and results and findings of the source tracking study.

Section 2
Study Area

The Trinity River Basin, found in the eastern one-half of Texas (Figure 2-1), has an overall length of approximately 350 miles. The basin's headwater region, found in North Central Texas, begins in Archer County with the West Fork. As it flows south-eastward, it is joined by the Clear Fork in Tarrant County and the Elm Fork in Dallas County to form the main stem, which is joined by the East Fork along the Kaufman/Ellis County line. The river then courses through East Texas to its mouth at Trinity Bay along the Gulf of Mexico. The total area drained by the Trinity River and its tributaries is approximately 18,000 square miles (TRA, 2003). The Upper Trinity Basin drains a large portion of North Central Texas, including the Cross Timbers and Prairies and the Blackland Prairie vegetational areas (Figure 2-2).

2.1 Cross Timbers and Prairies

The Cross Timbers stretch from Texas north to Kansas and are found in Texas from the Red River south for about 150 miles (Diggs et al., 1999). The Cross Timbers area of Texas consists of two distinct belts, the East and West Cross Timbers, which are separated by the enclosed Fort Worth Prairie (Figure 2-2). The East Cross Timbers area extends southward from the Red River through eastern Cooke and Denton Counties; continues along the Dallas-Tarrant County line, through Johnson and Hill Counties, into northern McLennon County. The West Cross Timbers extend from the Red River (Clay and Montague Counties) on the north, southward through Hood, Erath, Comanche and Brown Counties; and westward into Young, Stephens, and Callahan Counties.

The Cross Timbers, which are surrounded by prairie (Blackland Prairie to the east, Rolling Plains to the west), represent an ecotone between the eastern deciduous forest and the grasslands of the Great Plains (Diggs et al., 1999). The Cross Timbers is considered a mosaic of prairie, woodland, and savannah vegetation that is characterized by post oak (*Quercus stellata*) and blackjack oak (*Quercus marilandica*), the dominant overstory species. Understory dominants consist of little bluestem (*Schizachyrium scoparium*), big bluestem (*Andropogon gerardii*), and Indian grass (*Sorghastrum nutans*) (Dyksterhuis, 1948; Diggs et al., 1999; Hoagland et al., 1999). Invasion by mesquite and juniper trees, as well as a number of non-native species, have become an increasing problem by threatening the characteristic vegetation of the area and are a result of changing land use patterns, fire suppression, and past mismanagement by land owners (Diggs et. al., 1999). "One of the most striking features of the Cross Timbers is that this vegetational area contains significant remnants of ancient virgin forests" (Stahle and Hehr, 1984, cited in Diggs et al., 1999; Stahle et al., 1985, cited in Diggs et al., 1999), making it "one of the largest relatively unaltered forest vegetation types in the eastern United States" (Diggs et al., 1999).

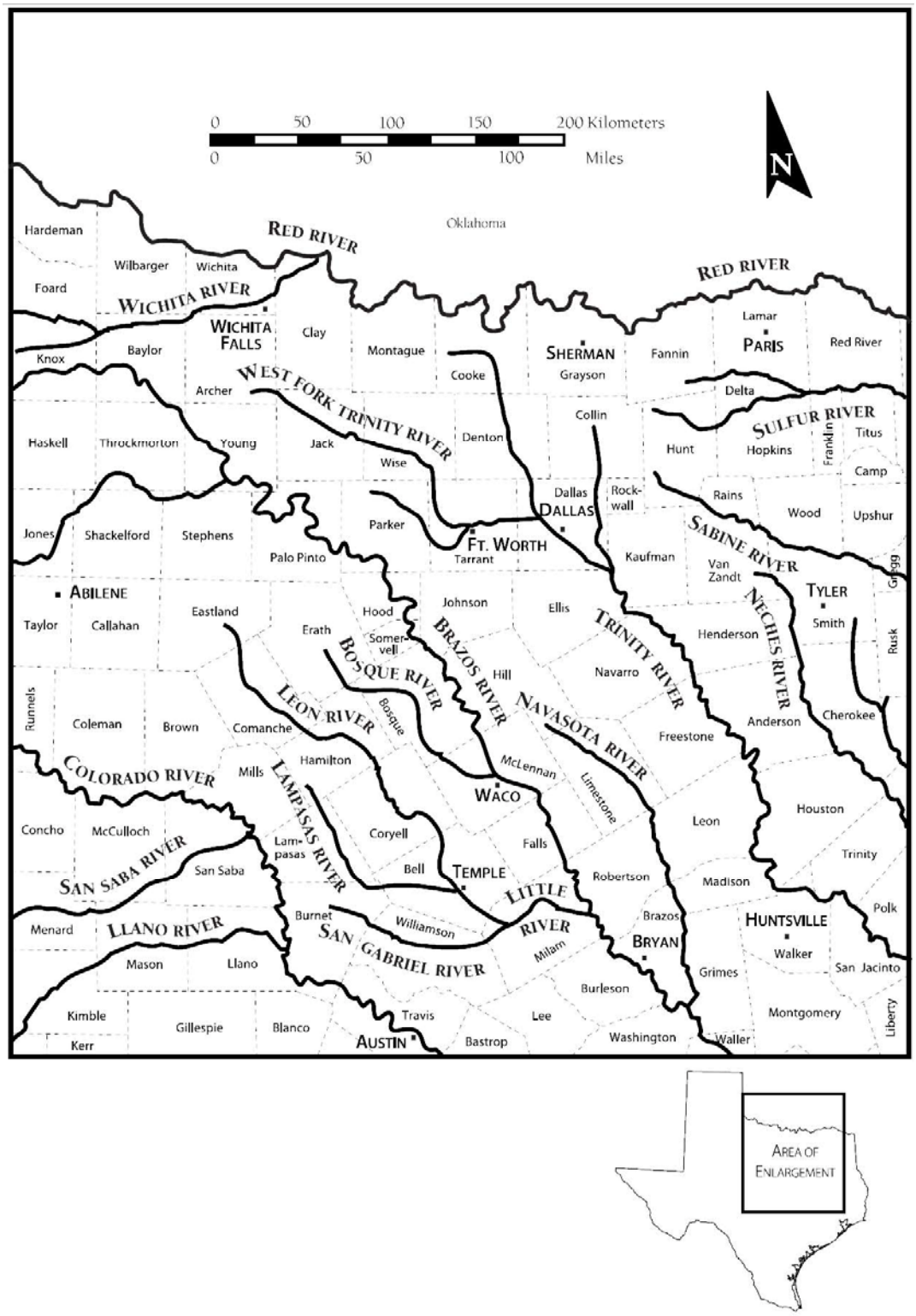

Figure 2-1. Major rivers of North Central Texas. Figure courtesy of Botanical Research Institute of Texas, Fort Worth.

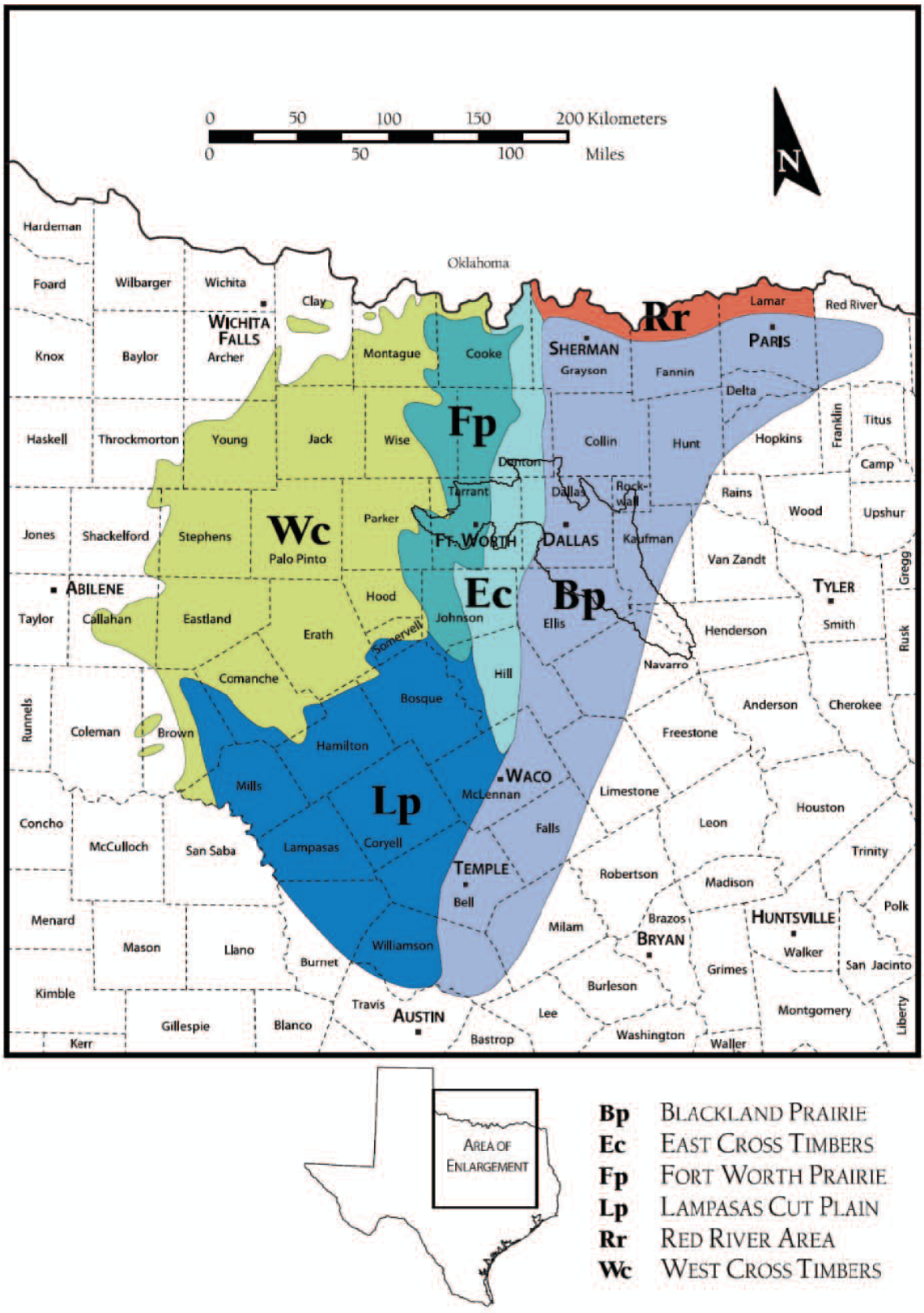

Figure 2-2. Vegetational areas of North Central Texas modified from Diggs et al. (1999) to show Upper Trinity River basin study area. Figure courtesy of Botanical Research Institute of Texas, Fort Worth.

The soils of the East and West Cross Timbers have primarily formed on sandy Cretaceous Woodbine and Trinity strata, with a small portion (western West Cross Timbers) having developed on rocky Pennsylvanian strata (Diggs et al. 1999). According to Diggs et al. (1999) the soils of the Cross Timbers are rather loose, often deep sands, which make them susceptible to erosion, but conducive to growth of the dominant woody vegetation (Diggs et al., 1999).

Agricultural activities generally consist of cattle operations (beef and dairy), horse farms, the cultivation of limited amounts of grain, hay, feed crops, and some fruit and nut production.

The **Fort Worth Prairie** is a 10- to 30-mile wide belt that separates the East and West Cross Timbers (Diggs et al., 1999). It is part of the larger Grand Prairie that extends from the Red River south to near Austin. The Fort Worth Prairie stretches south from the Red River in an irregular band through Cooke, Montague, Wise, Denton, Tarrant, Parker, Hood and Johnson Counties. The geology is predominantly a thin limestone soil on underlying limestone rock. The gently rolling plains around Fort Worth give way to rockier and steeper terrain as one travels south into the Lampasas Cut Plain portion of the Grand Prairie. Generally treeless, this area is dominated by prairie grasses such as little bluestem, side-oats grama (*Bouteloua curtipendula*), Indian grass, and big bluestem (Diggs et al., 1999). This area is primarily a livestock producing area, which includes beef and dairy cattle, horses, sheep and poultry. The majority of the crops grown in this area are for livestock feed, with some cotton grown as a cash crop (TRA, 2003).

2.2 Blackland Prairie

The **Blackland Prairie** region roughly extends 300 miles from the Red River in Grayson, Fannin, Lamar and Red River Counties south to near San Antonio in Bexar County (Diggs et al., 1999). This region covers approximately 10,600,000 acres, is slightly larger than the state of Maryland, and is widest in its northernmost range, narrowing to a point as it nears San Antonio (Collins et al., 1975, cited in Diggs et al., 1999). Geologically, the Blackland Prairie is located on an "erosional landscape that developed from easily erodible Cretaceous shales, marls, and limestones" (Hayward and Yelderman, 1991, cited in Diggs et al., 1999). According to Diggs et al. (1999), the Blackland Prairie is part of the True Prairie grassland, which extends from Texas north to Manitoba. "Based on climate, location, and vegetational characteristics, the Blackland Prairie can be considered part of either the True Prairie or Coastal Prairie associations" (Collins et al., 1975, cited in Diggs et. al., 1999). Its location allows it to be considered an ecotone between the True Prairie to the north and the Coastal Prairie to the south. Historically, much of the Blacklands were dominated by little bluestem communities in association with other dominants such as big bluestem and Indian grass. However, less widespread communities, dominated by eastern gamma grass (*Tripsacum dactyloides*), switchgrass (*Panicum virgatum*), tall dropseed (*Sporobolus compositus*), Texas cup grass (*Eriochloa sericea*), Florida paspalum (*Paspalum floridanum*), and long-spike tridens (*Tridens strictus*) were also found (Diggs et al., 1999)

Although this region was predominately prairie, some wooded areas were also found. These wooded areas consisted mainly of bottomland forests, wooded ravines along larger rivers and streams, mottes in protected areas, and woodlands found along contact zones with the

Edwards Plateau, Lampasas Cut Plain, and the Cross Timbers (Diggs et al., 1999). With the exception of preserves, small remnants, and native hay meadows, virtually nothing remains of the original Blackland Prairie communities, resulting in all of the tall grass community types being endangered or threatened (Diamond et al., 1987, cited in Diggs et al., 1999; Diggs et al., 1999). In addition, most of the region's native wetlands have also been lost. The most important cause resulting in the destruction of these ecosystems has been the conversion of the Blackland Prairie for agriculture (Diggs et al., 1999).

"Because of its early agricultural development the Blackland Prairie is still the most populated region in the state, containing within it and along its borders many of the state's largest and mid-sized cities," including Dallas, Waco, Austin, and San Antonio to name a few (TRA 2003). "As a result of the fertile soil and adequate rainfall, agricultural activity abounds in this area with cotton serving as the principal crop" (TRA 2003).

2.3 Land Use/Land Cover

The land use/land cover data were obtained from the 1992 Multi-Resolution Land Characteristic land use data by the U.S. Geological Survey (USGS, 1992). The total watershed area encompassing Segments 0806, 0841 and 0805 of the Trinity River Basin covers slightly more than 1,105,500 acres. For this study, the watershed area was defined as the contributing drainage of all tributaries below the most downstream dam of a major reservoir, if one exists. Since large reservoirs provide sufficient residence time to effectively remove a very high percentage of bacteria, areas above these reservoirs do not effectively contribute significant bacteria into the downstream study area. These major reservoirs include Lake Ray Hubbard on the East Fork, White Rock Lake on White Rock Creek, Grapevine Lake on Denton Creek, Lake Lewisville on the Elm Fork, Mountain Creek Lake on Mountain Creek, Lake Arlington on Village Creek, Lake Benbrook on the Clear Fork, Marine Creek Lake on Marine Creek, and Lake Worth on the West Fork.

Land use/land cover data are presented in an incremental drainage area manner for the 10 main-stem stations used in this BST study. The incremental drainage area for a particular station includes all tributary watersheds and main-stem drainage areas located between that particular main-stem station and the next upstream station. The most upstream station's incremental area continues to the upstream watershed boundary. (Note: greater descriptions of the 10 main-steam monitoring stations are provided in Section 3.1.1.) Using this incremental-area approach, the most immediate contributing drainage area of the 10 main-stem stations were individually determined allowing each stations immediate land use/land cover to be quantified. The land use/ land cover is represented by the following categories:

- **Improved Pasture**- Improved pasture is represented by land that has been converted into livestock grazing or hay production by replacing native forage with improved varieties or non-native species of grasses and/or forbs in order to increase forage production for livestock.

- **Residential**- Residential is property that has homes and/ or some other housing development present on it.

- **Forest**- Forest is land that contains a relatively high density of trees.

- **Cropland**- Cropland is land which has been cultivated and put into some sort of crop production. Crops may include small grains, row crops, orchards, vineyards, etc.

- **Commercial/ Industrial**- Commercial/ Industrial land is property that is occupied by commercial businesses, industrial complexes, and/ or transportational areas, such as highways, parking lots, or rail systems.

- **Open Water/ Wetlands**- Open Water/ Wetlands are areas that are occupied by rivers, streams, ponds, lakes, or reservoirs. These areas also include land that is inundated for periods long enough to develop wetland characteristics.

- **Native Pasture**- Native Pasture is land that is occupied by native grasses and/or forbs.

- **Turf**- Turf is land that is occupied by grasses suitable for parks, soccer fields, football fields, or other recreational complexes.

- **Shrubland**- Shrubland is land occupied by a high density of shrubbery.

- **Other**- Land categorized as "Other" includes bare rock, sand, or clay areas; quarries, stripmines, gravel pits, or other "transitional" areas.

Segment 0806

The Upper West Fork Trinity River (Segment 0806) has an incremental drainage area of 177,162 acres. Overall, Segment 0806 has dominant land uses consisting of 36% residential, 26% improved pasture, 14% native pasture and 11% commercial/ industrial property (Figure 2-3; Table 2-1). However, the drainage area above each of the segment's stream monitoring stations have their own specific land use/land cover distributions, which are discussed in more detail below.

Station 18459 – West Fork Trinity River, Segment 0806

Station 18459, located on the West Fork Trinity River at Northside Drive in Tarrant County, contains a little over 79,600 acres within its watershed. The majority of this watershed is occupied by residential areas (34%), native pasture (24%), improved pasture (18%), and commercial/ industrial (11%) land.

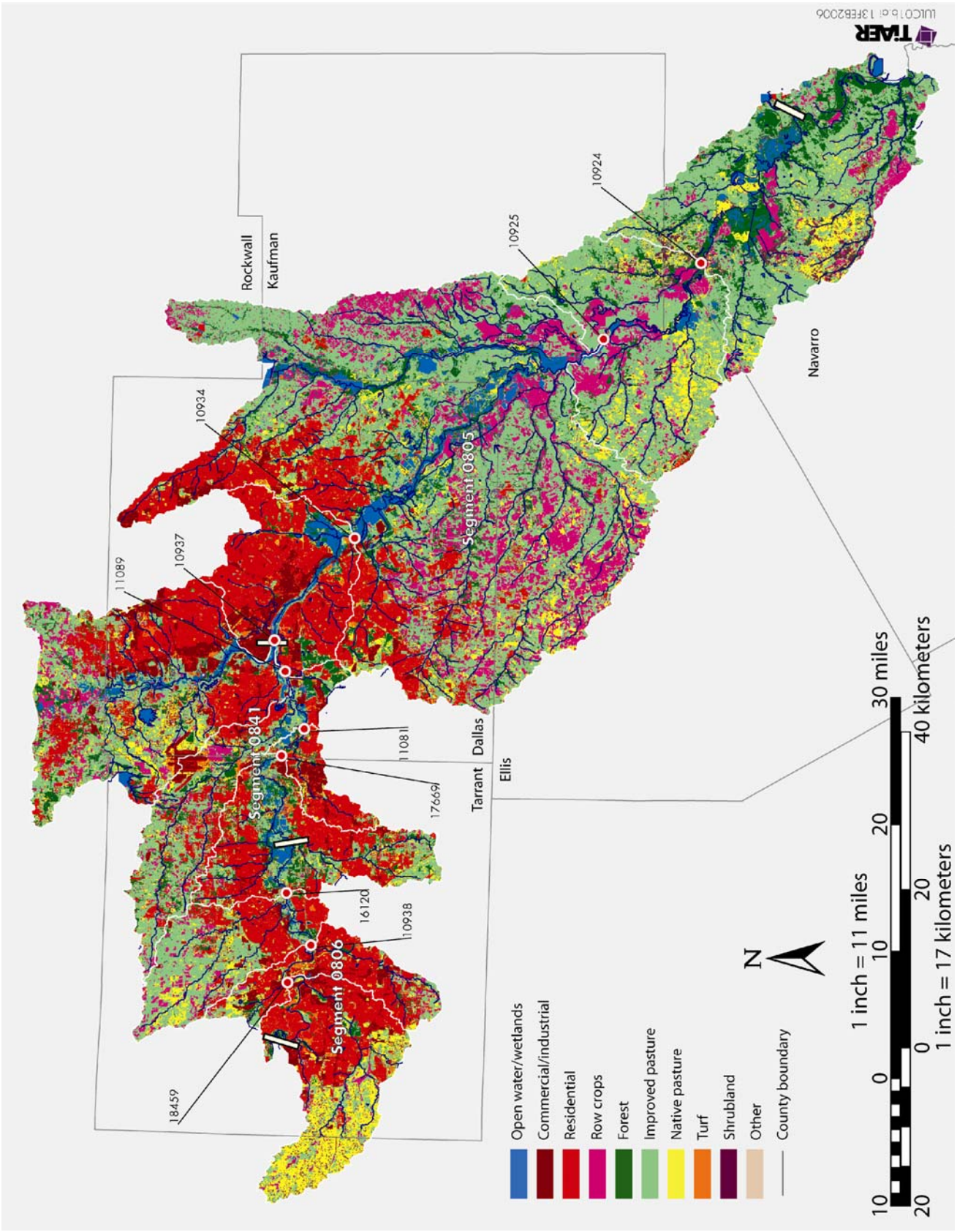

Figure 2-3. Land use/land cover data, presented in incremental drainage areas, for the Upper Trinity River basin.

Table 2-1. Land use/land cover summary and incremental contributing drainage area for stations associated with Segment 0806 of the Trinity River.

Description	18459	10938	16120	0806 Total
Residential (acres)	27322	20627	16240	64188
Residential (%)	34	53	28	36
Improved Pasture (acres)	14497	5942	25307	45746
Improved Pasture (%)	18	15	43	26
Native Pasture (acres)	19363	1629	3660	24651
Native pasture (%)	24	4	6	14
Commercial/ Industrial (acres)	8825	6642	4821	20287
Commercial/ Industrial (%)	11	17	8	11
Forest (acres)	3813	1328	2636	7776
Forest (%)	5	3	4	4
Cropland (acres)	1530	922	3560	6012
Cropland (%)	2	2	6	3
Turf (acres)	2269	1218	657	4144
Turf (%)	3	3	1	2
Open Water/ Wetlands (acres)	766	542	1596	2904
Open Water/ Wetlands (%)	1	1	3	2
Shrubland (acres)	1159	54	106	1319
Shrubland (%)	1	0	0	1
Other ((acres)	81	13	42	135
Other (%)	0	0	0	0
Total Acres	79623	38916	58623	177162
Total Percent (%)	100	100	100	100

Station 10938 – West Fork Trinity River, Segment 0806

Station 10938, located on the West Fork Trinity River at Beach St. in Tarrant County, has an incremental watershed area of almost 39,000 acres. Fifty-three percent of this watershed's land use consists of residential property, while commercial/ industrial (17%) and improved pasture (15%) lands are also major contributors.

Station 16120 – West Fork Trinity River, Segment 0806

Station 16120 is located on the West Fork of the Trinity River at Handley-Ederville in Tarrant County. Its incremental watershed contains approximately 59,000 acres. The majority of the land use in this watershed consists of improved pasture (43%) and residential (28%) property.

Segment 0841

The incremental drainage area of the Lower West Fork Trinity River (Segment 0841) is slightly smaller than Segment 0806, with a total of 163,194 acres. Its dominant land use categories consist of residential areas (35%), improved pasture (24%), forest (13%), and

commercial/ industrial (11%) (Figure 2-3; Table 2-2). The individual stations, making up this segment are discussed in more detail below.

Table 2-2. Land use/land cover summary and incremental contributing drainage area for stations associated with Segment 0841 of the Trinity River.

Description	17669	11081	11089	0841 Total
Residential (acres)	32185	9043	16267	57495
Residential (%)	43	52	23	35
Improved Pasture (acres)	14734	1132	23205	39071
Improved Pasture (%)	19	6	33	24
Forest (acres)	9096	972	11805	21873
Forest (%)	12	6	17	13
Commercial/ Industrial (acres)	7000	4234	7353	18587
Commercial/ Industrial (%)	9	24	10	11
Open Water/ Wetlands (acres)	7374	785	2859	11019
Open Water/ Wetlands (%)	10	4	4	7
Native Pasture (acres)	2327	370	2591	5289
Native pasture (%)	3	2	4	3
Turf (acres)	1883	881	1868	4632
Turf (%)	2	5	3	3
Cropland (acres)	645	22	3815	4482
Cropland (%)	1	0	5	3
Shrubland (acres)	301	47	260	608
Shrubland (%)	0	0	0	0
Other ((acres)	53	4	81	138
Other (%)	0	0	0	0
Total Acres	75597	17492	70105	163194
Total Percent (%)	100	100	100	100

Station 17669 – West Fork Trinity River at Roy Orr, Segment 0841

Station 17669 is located on the West Fork Trinity River at Roy Orr in Dallas County. The incremental watershed for station 17669 has a diverse association of land uses, containing over 75,000 acres. These lands are dominated by residential (43%) and improved pasture (19%), with forest and open water/ wetlands each contributing 12% and 10%, respectively.

Station 11081 – West Fork Trinity River, Segment 0841

Station 11081, located on the West Fork Trinity River at Belt Line Rd. in Dallas County, has an incremental drainage area over 17,000 acres. Residential areas comprise the largest portion (52%), followed by commercial/ industrial property, which represents 24%.

Station 11089 – West Fork Trinity River, Segment 0841

Station 11089, located on the West Fork of the Trinity River at West Loop 12 in Dallas County, contains approximately 70,000 acres in its watershed. Thirty-three percent of this

watershed is comprised of improved pasture land, while 23% consists of residential property, Forested land makes up 17% of the land cover, and 10% is commercial/ industrial.

Segment 0805

Upper Trinity River (Segment 0805) has an incremental drainage area of 765,205 acres, substantially larger than the previous two segments. It too has a diverse land use distribution, led by improved pasture land at 38%. Residential property occupies 20% of the total land use, followed by forest, with 12% and cropland, with 11% (Figure 2-3; Table 2-3). This diversity of land use/ land cover can be attributed to the upstream to downstream transition from dominance of urban land uses to rural uses (Table 2-4).

The individual stations making up this segment are discussed below.

Table 2-3. Land use/land cover summary and incremental contributing drainage area for stations associated with Segment 0805 of the Trinity River.

Description	10937	10934	10925	10924	0805 Total
Improved Pasture (acres)	39573	3025	169954	77228	289779
Improved Pasture (%)	25	4	42	59	38
Residential (acres)	47556	45036	57951	663	151205
Residential (%)	30	58	14	1	20
Forest (acres)	15067	3328	52967	18398	89759
Forest (%)	10	4	13	14	12
Cropland (acres)	8622	106	61457	14748	84932
Cropland (%)	6	0	15	11	11
Commercial/ Industrial (acres)	21717	15005	15202	673	52597
Commercial/ Industrial (%)	14	19	4	1	7
Open Water/ Wetlands (acres)	11405	6624	22538	6063	46630
Open Water/ Wetlands (%)	7	9	6	5	6
Native Pasture (acres)	7716	631	17334	11046	36727
Native pasture (%)	5	1	4	8	5
Turf (acres)	3492	3320	3392	144	10348
Turf (%)	2	4	1	0	1
Shrubland (acres)	854	63	643	1388	2948
Shrubland (%)	1	0	0	1	0
Other ((acres)	84	5	126	63	278
Other (%)	0	0	0	0	0
Total Acres	156085	77142	401564	130414	765205
Total Percent (%)	100	100	100	100	100

Station 10937 – Trinity River, Segment 0805

Station 10937, located on the Trinity River at Mockingbird Lane in Dallas County, has an incremental drainage area containing over 156,000 acres. The majority of this watershed consists of residential property and improved pasture, each contributing 30% and 25%, respectively. Commercial/ industrial land represents 14%, while forest land represents 10%.

Table 2-4. Land use/ land cover differences between the upper and lower two stations of Trinity River Segment 0805.

Description	Upper	Lower
Improved Pasture (acres)	42,598	247,181
Improved Pasture (%)	18	46
Residential (acres)	92,591	58,614
Residential (%)	40	11
Forest (acres)	18,394	71,365
Forest (%)	8	13
Cropland (acres)	8,728	76,204
Cropland (%)	4	14
Commercial/ Industrial (acres)	36,722	15,875
Commercial/ Industrial (%)	16	3
Open Water/ Wetlands (acres)	18,029	28,601
Open Water/ Wetlands (%)	8	5
Native Pasture (acres)	8,346	28,380
Native pasture (%)	4	5
Turf (acres)	6,813	3,536
Turf (%)	3	1
Shrubland (acres)	917	2,031
Shrubland (%)	0	0
Other ((acres)	89	190
Other (%)	0	0
Total Acres	233,227	531,978
Total Percent (%)	100	100

Station 10934 – Trinity River, Segment 0805

Station 10934 is located on the Trinity River at South Loop 12 in Dallas. The watershed for station 10934 contains approximately 77,200 acres. The majority (58%) of this watershed is residential and 19% is commercial/ industrial.

Station 10925 – Trinity River, Segment 0805

Station 10925 is located on the Trinity River at SH34 near the town of Ennis in Ellis County. The watershed for station 10925 contains over 401,000 acres. Forty-two percent of this watershed is improved pasture, 15% is made up of cropland, 14% is residential, and 13% is forest.

Station 10924 – Trinity River Segment 0805

Station 10924, located in Navarro County, is on the Trinity River at its intersection with FM85. The watershed for station 10924 contains approximately 130,000 acres. Fifty-nine percent of the land use in this watershed is represented by improved pasture, 14% is represented by forest, and 11% is cropland.

2.4 Estimated Human, Pet and Livestock Populations in Watershed Counties.

The estimated population data for humans, pets and various livestock species present in counties that are drained by the West Fork and Upper Trinity River watersheds were obtained from the U.S. Census Bureau, the American Veterinary Medical Association, the USDA National Agricultural Statistics Service, and the Texas Agriculture Statistics Service (Table 2-5).

2.5 Climate

North Central Texas has a subtropical climate characterized by hot summers and mild winters, resulting in a wide annual temperature range (National Weather Service (NWS), 2005). Average high temperatures generally reach their peak of 96°F between late July and mid August. Fair skies generally accompany the highest temperatures of summer, which are often above 100ºF; however, the low temperature rarely exceeds 80ºF at night (NWS, 2005). During winter, the average low temperature bottoms out at 33ºF in early to mid January and periods of extreme cold generally do not last long (NWS, 2005). The frost-free period generally lasts for about 248 days, with the last frost occurring in mid March and the first frost occurring in mid to late November (NWS, 2005).

Precipitation, like temperatures of the area, has a tendency to vary considerably. Yearly average precipitation ranges from 24 inches in the western areas of North Central Texas to 46 inches in the northeast areas. According to Bomar (1983), "In general, mean annual precipitation decreases about one inch for each 15 miles across Texas from east to west "(cited in Diggs et al., 1999). Diggs et al. (1999) states "severe storms and some of the largest rainfalls in the United States have occurred in this area." "All the point rainfall records for North America are held within a belt 50 miles east and west of a line from Dallas through Waco, Austin, and San Antonio"(Hayward et al., 1992, cited in Diggs et al., 1999). Rainfall in the area generally occurs at night, with most of the thunderstorms occurring during the spring. Snowfall in this area is a rare occurrence (NWS, 2005).

2.5 Hydrology and Water Quality Issues of the Basin

"Generally, streamflow in the Trinity River Basin follows the rainfall pattern of the area" (TRA, 2003). Although the Trinity River Basin has moderate rainfall and runoff on average, its hydrology is notoriously erratic, ranging from floods to drought. During normal years much of the rain and streamflow occur in late spring, followed by very hot, dry weather from mid-June through August, into September (TRA, 2003). According to the Trinity River Authority (2003), "the natural flow in the great majority of streams in the Trinity Basin is highly variable" and "most of the smaller streams in the basin cease to flow within a few days or weeks without rain, depending on the season and drainage area." Many of the Trinity River's tributaries, and the river itself below Dallas, have a base flow which consists mainly of effluent discharged from wastewater treatment plants. "Extensive sampling and monitoring have proven that more than 90% of the river's flow, below Dallas, during periods of dry weather originates from the wastewater treatment plants of Fort Worth, Dallas, Garland, and the Trinity River Authority" (TRA 2003). These streams, or portions of streams, are often referred to as being "effluent

Table 2-5. Estimated population data for humans, pets and various livestock and wildlife species present in counties that are drained by the West Fork and Upper Trinity River watersheds.

Category	Source	County									
		Collin	Dallas	Denton	Ellis	Henderson	Kaufman	Navarro	Parker	Rockwall	Tarrant
Avian	Pigeons °	199	N/A	74	280	25	97	178	348	60	N/A
Avian	Poultry (Ducks, Geese, Other) °	2001	1658	4361	5068	1581	2780	1513	2597	487	2444
Human	(Households)*	181,970	807,621	158,903	37,020	28,804	24,367	16,491	31,131	14,530	533,864
Human	Humans*	627,938	2,218,899	530,597	128,710	79,184	85,377	48,243	100,336	58,260	1,588,088
Livestock	Bison °	N/A	N/A	73	408	57	N/A	6	35	N/A	73
Livestock	Cattle (All) ‡	42,000	9,000	51,000	53,000	76,000	87,000	82,000	69,000	7,000	19,000
Livestock	Goats ‡	6,000	1,100	3,000	3,200	1,300	2,100	2,700	6,900	N/A	1,000
Livestock	Hogs/ Pigs °	374	728	165	333	501	613	750	781	57	944
Livestock	Horses/ Ponies°	4,779	2,032	9,517	3,443	3,476	5,155	2,419	9,379	1,068	3,676
Livestock	Mules/ Burros/ Donkeys °	274	70	333	359	268	447	253	635	31	110
Livestock	Poultry (Chickens &Turkeys) °	2393	970	347	6522	1324	6801	3442	6451	978	2932
Livestock	Sheep ‡	N/A	N/A	1,000	1,000	N/A	N/A	N/A	N/A	N/A	N/A
Mammalian Wildlife	Deer (Domestic) °	29	12	N/A	160	303	N/A	210	114	N/A	435
Mammalian Wildlife	Rabbits °	220	1,895	64	145	85	677	735	372	7	200
Pet	Cats †*	146	1,258	42	96	56	449	488	247	5	133
Pet	Dogs †*	127	1,095	37	84	49	391	425	215	4	116

° = based on 2002 USDA Census of Agriculture-County Data; National Agriculture Statistics Service

‡ = based on 2005 Texas Agricultural Statistics Service

† = based on 2001 statistics from *2002 U.S. Pet Ownership and Demographic Sourcebook*, American Veterinary Medical Association

*** = based on U.S. Census Bureau's Online State and County Quickfacts; accessed 5/11/2006.**

dominated." With regard to the Trinity River, "the biggest effluent dominated reach is the main stem from Dallas and Fort Worth to Lake Livingston. In dry weather the flow in this reach is almost entirely made up of discharges from wastewater treatment plants (WWTP)" (TRA, 2003). In addition, many of the smaller streams in the Dallas/Fort Worth area have a small base flow which consists of point and nonpoint discharge from various sources. The TRA (2003) states "these streams may have poor quality at base flow, as well as the leading edge of a rise. Dissolved oxygen is occasionally low and bacteria are often very high." Potential sources of nonpoint source pollution include "overflows from wastewater collection systems, septic system leakage, leachate from solid waste facilities, construction activities, and agricultural operations" (TRA, 2003).

Section 3
Materials and Methods

In order to carry out the stated objectives, the study had to be broken down into varying stages. These included:

- Station Selection
- BST Water Sampling
- Known Source Fecal and Sewage Ribotype Library Development
- Ribotyping of BST Water and Known Source Fecal and Sewage Samples

3.1 Station Selection

Station selection for BST water sampling started with the objective to identify the sources contributing to violations in water quality criteria for bacteria in the three segments of the Trinity River. A number of factors were taken into consideration prior to selecting monitoring stations. A particularly important factor was that the confidence in source estimates depended on the number of isolates matched from ambient samples to known fecal samples. To get reasonably accurate estimates, past project experience indicated a need for a minimum of 70 – 120 isolates per station, with more being better. This study was designed for sampling at 10 main-stem stations with collection of 110 isolates per station in order to provide a reasonable database of isolates.

Another important factor was that the stations needed to be properly assigned to their corresponding segments in order to adequately characterize various reaches and to isolate, whenever possible, major contributing areas. Historical data from most of the main-stem stations indicated high bacteria concentrations that exceeded criteria. For some stations, however, the selection was based on location within an impaired reach, allowing better characterization of the upstream and downstream portions of the reach regardless of the presence of historical data or a history of bacteria impairment.

After consideration of the various factors involved, the 10 stations were chosen for monitoring. Segment 0806 had three main-stem stations (18459, 10938, and 16120), Segment 0841 also had three main-stem stations (17669, 11081, and 11089), and Segment 0805 had four main stem stations (10937, 10934, 10925, and 10924) **(**Figure 3-1**)**.

3.1.1 Monitoring Station Descriptions

A brief description of each station, monitoring activities, and the purpose for station selection are provided below:

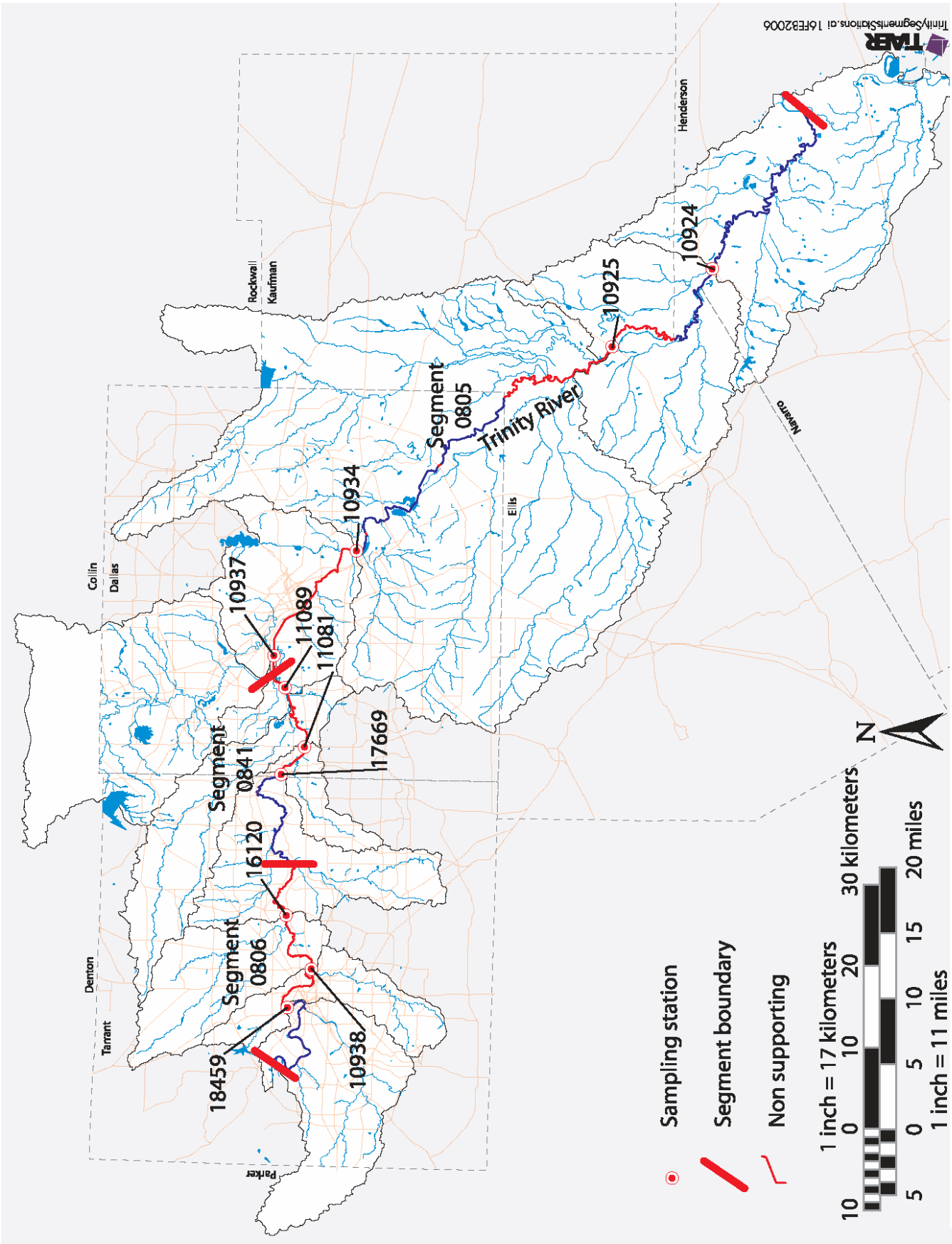

Figure 3-1. Trinity River study area showing BST monitoring stations.

Segment 0806 – West Fork Trinity River below Lake Worth

Station 18459 was located on the West Fork Trinity at W. Northside Dr. in north Fort Worth near the Stockyards. This station was selected because it was the upper most accessible site on the impaired portion of Segment 0806 that was a sufficient distance below the confluence of the Clear Fork and West Fork for mixing of these two streams to occur.

Station 10938 was located on the West Fork Trinity River at Beach Street in Fort Worth. USGS stream gauge 08048543 is located at this site. This station was located near the upstream end of the impaired assessment unit in Segment 0806

Station 16120 was located on the West Fork Trinity River at Handley-Ederville Road approximately 0.55 km upstream of IH 820 in Fort Worth. This station was located near the middle of the impaired assessment unit in Segment 0806.

Segment 0841 - Lower West Fork Trinity River

Station 17669 was located on the Lower West Fork Trinity River at Roy Orr Blvd. in Grand Prairie. USGS stream gauge 08049500 is located at this site. This station was located immediately upstream of an assessment unit that showed nonsupport of contact recreation, and recent bacteria samples from 2000-2004 showed concentrations exceeding pertinent criteria.

Station 11081 was located on the Lower West Fork Trinity River at Beltline Road in Grand Prairie. This station was located in an assessment unit that showed nonsupport of bacteria criteria.

Station 11089 was located on the Lower West Fork Trinity River at West Loop 12 in Irving. This station was located near the downstream end of an assessment unit that showed nonsupport of bacteria criteria.

Segment 0805 - Upper Trinity River

Station 10937 was located on the Upper Trinity River at Mockingbird Lane southwest of downtown Dallas. This station was located in an assessment unit that showed nonsupport of bacteria criteria.

Station 10934 was located on the Upper Trinity River at South Loop 12 below Dallas. USGS stream gauge 08057410 is located at this site. This station was located in an assessment unit that showed nonsupport of bacteria criteria.

Station 10925 was located on the Upper Trinity River at State Highway (SH) 34 at the Ellis/Kaufman County line northeast of Ennis. USGS stream gauge 08062500 is located at this site. This station was located in an assessment unit that showed nonsupport of bacteria criteria.

Station 10924 was located on the Upper Trinity River at Farm Road (FM) 85 along the Henderson/Navarro County line west of Seven Points. This station was selected because it is the most downstream station of Segment 0805 that is accessible for monitoring.

3.2 BST Water Sampling

Water sampling for bacteria had two purposes: 1) collect quantitative data for *E. coli* concentrations in the Trinity River, and 2) primarily to collect *E. coli* isolates for subsequent BST analysis.

3.2.1 BST Water Sample Collection and Laboratory Procedures

BST water sampling events for *E. coli* were conducted on approximately a two per month basis beginning in March 2005 and lasting through August 2005. These samples were collected at 10 main-stem stations from the West Fork of the Trinity (3 stations each for stream Segments 0806 and 0841) and the Upper Trinity River (4 stations from stream Segment 0805) basins**.** Because *E. coli* populations have been found to vary on fine spatial and temporal scales, sampling representativeness during BST water sampling was increased by collecting five independent water samples equally spaced across the stream, one to five minutes apart, at each of the 10 main-stem stations. This sampling was performed on 11 separate events for a total of 550 water samples. Two *E. coli* isolates per sample were then submitted for to IEH for ribotyping, for a total of 1,100 isolates.

The Project Team followed the field sampling procedures documented in the TCEQ *Surface Water Quality Monitoring (SWQM) Procedures Volume 1: Physical and Chemical Monitoring Methods for Water, Sediment and Tissue* (December 2003). Bacteria water samples were collected directly from the stream (up to one-foot below the surface) into reusable sterile polypropylene bottles, in which 0.25 ml of 10% solution of sodium thiosulfate (per 250 ml of sample collected) was previously added to be used where chlorination was suspected. Care was exercised during sample collection to avoid the surface microlayer of water, which may be enriched with bacteria and not representative of the water column. In cases where, for safety reasons, it was inadvisable to enter the stream bed, the Project Team sampled from a bridge using a weighted PVC sampler that was designed to hold a sample container. After collection each sample container was then labeled with an indelible, waterproof marker. Label information included station identification, date, and time of sample collection. Field sampling personnel wore clean, disposable gloves when they were likely to come in contact with samples that may become contaminated.

Following sample collection, samples were placed into Ziploc® style bags, in order to further prevent contamination, and carried on ice in ice chests from the point of collection to the TIAER mobile laboratory, which was located in a central location of the study area[1]. The TIAER lab staff received the samples, a copy of the chain of custody, and copies of the field data sheets. The lab staff then logged the individual sample information into the laboratory log book. This information included a unique sample ID, test group code, station name, date of collection,

[1] The TRA allowed TIAER to locate its mobile laboratory at a secure location of an abandoned pump station near monitoring station 11089.

time of collection, project name, collector's name(s), and receiver's name. After logging the sample information into the logbook, the samples were checked for proper temperature. The acceptable temperature range was 0-6 °C. If the temperature exceeded 6°C, the lab documented whether or not wet ice was present. If ice was present, the temperature variation was considered to be attributed to a short holding time that did not allow the samples to cool to between 0°C and 6°C. Samples were considered acceptable if they were placed on ice immediately after collection, remained on ice upon receipt by the laboratory, sample preparation was initiated within six hours after the sample was collected, and sample preparation was completed within eight hours of collection.

The TIAER lab staff utilized the membrane filter technique for *E. coli* using modified mTEC agar. Upon sample preparation 0.1, 1.0, 10.0, and 100 ml of the sample solution were filtered through a 0.45 micron nitrocellulose filter. The filter was then transferred to a Petri dish containing modified mTEC agar, inverted and incubated for 2 hours at 35.0 +/- 0.2 °C to rehabilitate injured colonies, then an additional 22-24 hours at 44.5 +/- 0.2 °C. Following the 22-24 hour culture incubation and subsequent concentration enumeration, one Petri dish per sample, containing approximately 20-80 *E. coli* colonies, was chosen for BST analysis. The cultures were labeled appropriately and transferred to an insulated shipping container with blue-ice cold packs for cooling. A member of the TIAER lab staff then enclosed the sample chain of custody in the shipping container and sent it via overnight courier to IEH in Seattle, WA for ribotyping and comparison to known source samples.

3.3 Known Source Fecal and Sewage Ribotype Library Development

3.3.1 Sanitary Survey

A key component of the monitoring plan was preparation of a sanitary survey for the West Fork Trinity River and Upper Trinity River watersheds. Through the sanitary survey potential sources and general categories of fecal contamination within the watershed were identified and listed, thus ensuring that collection and analysis of resident *E. coli* strains from each known contributing source was accomplished. Identified sources included various wildlife, livestock, avian, and pet species, as well as Human influences. Based on information obtained from the sanitary survey, a field collection strategy was defined for collecting known fecal source samples from throughout the watershed, including a list of target species and a recommended number of fecal samples to collect from each species (Table 3-1).

In addition to the sanitary survey, the Project Team reviewed available literature, data, and information pertinent to describing the contributions and defining sources of bacterial loading in the watersheds. Special emphasis was placed on acquiring land use/land cover, human/agricultural census data, and wastewater/ storm water infrastructure. These data were integral in assisting in the planning and execution of the project. Several other types of existing data and information were useful in the sanitary survey. These data included:

- reported wastewater permit information, including permit limits, self-reported effluent quality data, violations, and inspection reports;

Table 3-1. Summary of target and realized fecal source sampling for library development.

Major Category	Minor Category	Target No. Samples to Collect	Total Samples Collected	Total Percentage of Target Sampled
Avian	Pigeon	20	17	
	Swallow	20	14	
	Other	20		
	(Heron)	-----	1	
	(Grackle)	-----	21	
	(Egret)	-----	10	
	(Kingbird)	-----	1	
	(Sparrow)	-----	4	
	(Dove)	-----	8	
	(Red-winged Blackbird)	-----	1	
	(Flycatcher)	-----	1	
	(Starling)	-----	3	
	(Vulture)	-----	3	
	(Killdeer)	-----	2	
	(Seagull)	-----	2	
	Roadrunner	-----	1	
	Guinea	-----	2	
	Total	**60**	**91**	**152%**
Human	Human (Raw Sewage	35	58	
	Human (Septage)	35	20	
	Human (WWTP Effluent)	0	10	
	Total	**70**	**88**	**126%**
Mammalian Wildlife	Armadillo	0	1	
	Bobcat	0	2	
	Deer	10	0	
	Mouse	5	0	
	Opossum	5	7	
	Rabbit	5	5	
	Raccoon	10	5	
	Rat	5	12	
	Squirrel	5	5	
	Other	15	0	
	Total	**60**	**37**	**62%**
Livestock	Cattle (Dairy)	15	14	
	Cattle (Beef)	45	65	
	Bison	0	6	
	Poultry	45	59	
	Horse/Donkey	10	25	
	Goat	5	19	
	Sheep	5	6	
	Llamas	0	3	
	Pig (Domestic)	5	9	
	Total	**130**	**206**	**158%**
Pets	Cat / Feline	30	23	
	Dog / Canine	30	61	
	Other	10	16	
	Total	**70**	**100**	**143%**
Petting Zoo	Various Species	10	0	
	Total	**10**	**0**	**0%**
All Species	**Grand Total**	**400**	**522**	**131%**

- hydrologic and meteorological data;
- the extent to which on-site sewage systems (septic tanks) are used in the watershed;
- estimated populations of domestic pets; and
- special studies and published reports for the study area.

3.3.2 Known Source Fecal and Sewage Ribotype Library Sample Collection

For the known source fecal and sewage sampling, the Project Team field staff collected specimens directly from the source feces, with the exception of human samples, which were collected from WWTP influent**.** In some cases, wildlife samples were collected indirectly from "found" fecal samples. The sources of these "found" wildlife fecal samples were identified to the lowest practical taxonomic level by experienced field biologists**.** In cases of uncertainty regarding its source, the sample was not used for library development. No more than 15 samples were collected from the members of the same animal species from a given location, unless those animals did not normally comprise a distinct population of low diversity, but were assembled temporarily (e.g., a livestock show, animal shelter, or migrating waterfowl). Only a single sample was collected from an individual animal. The overall source-sampling objective is to collect several, representative samples from every warm-blooded species known to populate the watersheds in sufficient numbers to be a potential source of the documented bacterial contamination.

Fresh animal fecal samples were collected aseptically, using a sterile spatula or swab, into sterile, screw-cap polypropylene specimen tubes, which were then capped and sealed. All sample containers were then labeled with the following information:

- sample type,
- host species,
- collection date,
- collection time,
- sample location, and
- sampler's initials.

All of the sample information was logged into a field logbook or noted on field data sheets. Samples were immediately placed into a cooler on wet ice in double zip lock bags and later transferred to a shipping container with blue-ice cold packs and shipped via overnight courier to IEH for ribotyping. Human wastewater samples were first plated for growth of *E. coli* colonies and then shipped (using the same cooling protocols) to IEH for ribotyping.

3.3.3 Ribotyping of BST Water and Known Source Fecal and Sewage Samples

Upon receipt of the known source fecal and sewage samples by IEH, one *E. coli* isolate from each Petri dish sample was scraped and prepared for DNA extraction. Following DNA extraction, the DNA samples were digested using *Eco*R1 and *Pvu*II endonuclease restriction

enzymes. The resulting DNA fragments were then run on a 0.8% agarose gel and subsequently stained using ethidium bromide. The gel was then photographed and labeled. Label information included the isolate numbers loaded on each lane, the enzyme used to cut the DNA, date, gel number, voltage, current, gel strength, buffer strength, and electrophoresis time information.

The gel was then processed for Southern Blot Hybridization. The resulting ribotype was then analyzed based on the distance between the DNA bands. The ribotypes were then entered into a Microsoft Access® database and compared to ribotypes of known source (Complete IEH Ribotyping Protocol may be found in Appendix I).

3.4 Additional Data Collection

3.4.1 Anecdotal Record

The anecdotal record consists of a written log of field observations made at each station on the segment during monitoring activities. This record describes the physical stream characteristics noted by the Project Team field staff. At the time of water sample collection, all field observations were recorded in a field data logbook or on field data sheets. The data included, but were not be limited to: the name of sampler(s), date, time, station identification, depth of sample, qualitative estimation of flow severity, water body type, water appearance, weather conditions, days since last significant rainfall, stream uses, unusual riparian conditions, odors and any other significant sample information (as applicable). (Selected anecdotal and *E. coli* quantification data may be found in Appendix II.)

3.5 Schedule of Sampling Events

3.5.1 BST Water Sampling

BST water sampling occurred on each of the following dates:

- 23 March 05 (all stations; event 1)
- 05 April 05 (all stations; event 2)
- 19 April 05 (all stations; event 3)
- 04 May 05 (all stations; event 4)
- 17 May 05 (all stations; event 5)
- 01 June 05 (all stations; event 6)
- 14 June 05 (all stations; event 7)
- 28 June 05 (all stations; event 8)
- 12 July 05 (all stations; event 9)
- 27 July 05 (all stations; event 10)
- 08 August 05 (Stations 10937, 10934, 10925, and 10924; event 11)
- 16 August 05 (Stations 18459, 10938, 16120, 17669, 11081, and 11089; event 11)

3.5.2 BST Water Sampling Event Summary

Using data from the anecdotal record, a sampling event summary was developed in order to provide information concerning atmospheric, hydrologic, or other site specific information. This summary provides additional data in order to help explain any potential anomalies that may be discovered at a later date. The sampling event summary includes the following events:

Event 1

The first water sampling event occurred on March 23, 2005. The weather was clear to partly cloudy with a slight to moderate breeze, generally out of the north. Hydrologic conditions at each of the stations appeared to be normal since no significant rainfall had fallen for over a week. With the exception of a slight chlorine odor and some patches of foam at station 11089, there were no particularly unusual conditions to report.

Event 2

The second sampling event took place on April 5, 2005. The weather on this particular day was cloudy early in the morning; changing to clear to partly cloudy skies later in the morning and into the afternoon. The wind was a slight to strong southerly breeze and the stream hydrologic conditions were found to be normal, as there were no significant rainfall accumulations during the previous seven days. With the exception of swallows present under the bridge at station 10925, there were no remarkable conditions to report.

Event 3

The third sampling event occurred on April 19, 2005. The weather was found to be a cloudy day with slight to strong south/southwest wind. Stream flow conditions were generally normal since no significant rain had fallen during the previous seven days. Station 10924 in Segment 0805 had a slight sheen present on the surface of the water and the water was notably turbid during this sampling event. Swallows were found to be present under the bridges at most of the stations in Segments 0806 and 0841.

Event 4

The fourth sampling event took place on May 04, 2005. The weather was cloudy with rain being observed at station 10924, and the wind was calm to slight. Despite the fact that significant rain was recorded during the previous 24 hours, the stream hydrologic conditions were still considered normal. No other remarkable conditions were observed at any of the stations.

Event 5

The fifth sampling event occurred on May 17, 2005. The weather was clear to partly cloudy with calm to moderate winds out of the south. Despite a significant rain event taking

place three days prior to sampling, the hydrological stream conditions appeared normal. A large number of cliff swallows were observed at the bridges of stations 10938 and 17669.

Event 6

The sixth sampling event occurred on June 01, 2005. The weather on this day was clear to partly cloudy with a slight to moderate northwesterly wind. The last significant rainfall occurred within 24 hours of this sampling event, resulting in elevated flows and turbid water observed at many of the upper stations.

Event 7

The seventh sampling event took place on June 14, 2005. The weather was clear to partly cloudy with calm to moderate winds out of the northwest. The last significant rain event occurred over a week prior; this coupled with warming temperatures resulted in low flows being observed in much of the Upper Trinity. No other remarkable conditions were observed during this sampling run.

Event 8

The eighth sampling event, on June 28, 2005, was conducted under clear to partly cloudy weather conditions with calm to slight southwesterly winds. Hydrologic conditions in the stream were found to be low to normal since a significant rainfall event had not taken place since June 1. A large number of swallows at station 10938 and a Jeep located in the river upstream of our sampling station at 16120 were the only remarkable conditions observed during this event.

Event 9

The ninth sampling event took place on July 12, 2005. The weather was clear to partly cloudy with calm to slight winds out of the south. Stream hydrologic conditions were low to normal. The last significant rain fell five days prior. No other remarkable conditions were noted.

Event 10

The tenth sampling event, occurring on July 27, 2005, took place during rainy weather, with a slight northwest wind. Stream conditions were found to be normal despite the rain. No other remarkable conditions were observed.

Event 11

The eleventh and last sampling event took place over two days, each about a week apart. On August 08, 2005, the stations in Segment 0805 were sampled. This day was cloudy with a moderate wind. Streamflow was found to be high since a significant rain had fallen less than 24 hours prior. No other remarkable conditions were noted. Segments 0806 and 0841 were sampled the following week on August 16, 2005. During this sampling event, the weather was

found to be partly cloudy with a slight south wind. Since a significant rainfall event occurred less than 24 hours prior to this sampling event, the stream hydrologic conditions were found to be high. No other remarkable conditions were found to exist.

3.5.3 Known Source Fecal and Sewage Ribotype Library Sample Collection

Known source fecal and sewage ribotype library sample collection was also performed on approximately a two per month basis throughout the six-month study in order to ensure representative sampling of as many human, wildlife, and domestic animal sewage/fecal specimens as possible. Known source fecal and sewage ribotype library sample collection occurred on the following dates:

- 23 March 05
- 19 April 05
- 17 May 05
- 14 June 05
- 07 July 05
- 08 August 05

05 April 05
04 May 05
01 June 05
28 June 05
12 July 05
16 August 05

Section 4
Results

4.1 Quality Assurance/Quality Control Results

QA/QC measures utilized by TIAER in the culturing and enumeration of *E. coli* from water samples, and by IEH in the ribotyping of *E. coli* are described separately below.

4.1.1 Culturing and Enumeration of *E. coli*

TIAER laboratory personnel carried out QA/QC procedures in order to: 1) verify the sterility of the reusable *E. coli* collection containers, media, filters, glassware, and other supplies and equipment. through analysis of method blanks, 2) quantify any variation in the analytical process through analysis of laboratory duplicates, and, 3) determine the viability of the bacterial growth media by running a positive and negative control sample on each new batch of growth media that was produced.

The sterility of the reusable *E. coli* containers, media, filters, glassware, and other supplies and equipment was verified by analyzing a method blank. This procedure involved adding approximately 250 ml of sterile deionized water to a sterilized re-usable container before analysis on each day that project samples were collected and for every set of 20 samples that were processed, a 100 ml sample of water was pulled from the method blank and also processed by carrying it through the entire analytical procedure.

Laboratory duplicates were used in order to quantify any variation in the analytical process. This procedure involved analyzing an additional aliquot from a sample bottle for every 10 samples that were processed. This additional sample was then carried through the entire analytical procedure. (Data from culturing and enumeration QA/QC procedures may be found in Appendix III.)

Positive and negative control cultures were also processed with each batch of bacterial growth media that was produced. Positive controls involved inoculating a sterilized Petri dish containing the fresh sterilized media with a positive *E. coli* sample in order to verify that it would grow on the media. Negative controls involved inoculating a sterilized Petri dish containing the fresh sterilized media with a bacterial species other that *E. coli* (usually *Pseudomonas sp.*) in order to verify that it would not grow on the media. All positive controls were positive for the growth of *E. coli* and all negative controls were negative for the growth of any extraneous species.

4.1.2 Ribotyping QA/QC Results

BST does not lend itself easily to the same QC methods as chemical quantification. Blank samples may be irrelevant, and replicate water samples may often yield different *E. coli* strains. The method accuracy and precision was quantified through a special QC study with "double-blind" safeguards, as practiced in epidemiological QC.

IEH prepared triplicate cultures of 30 *E. coli* isolates from known sources collected from the Trinity River segments of interest. These isolates were selected by Parsons and TIAER from a variety of species in the known source library. The 90 (30 x 3) cultures were then placed in 90 identical culture tubes, each with a removable label indicating their source and the isolate number. These tubes were mailed to the Parsons Project Manager (PM). The Parsons PM prepared and sent a list of the 30 isolate sources to the TIAER PM, who selected, from the list, 10 isolates to be blind QC test samples. By selecting a subset of only 33% of the prepared cultures, the laboratory had no basis for anticipating the identity of the unmarked samples that they received. The Parsons PM then identified the 30 culture tubes associated with those 10 isolates, replaced each label with a new label, numbered them from 1 to 30 in random fashion, and recorded those numbers on a key with the isolate number and source. The Parsons PM then sent those 30 culture tubes back to IEH after verifying that there was no way for their source to be identified. The Parsons PM sent the key to the TIAER QAO and PM. The samples were processed through the ribotyping procedures in a blind fashion; that is the laboratory did not know the sources. IEH then sent the results to the TIAER PM, who made a copy of the key and results and provided it to IEH and the Project Team QAO. IEH successfully ribotyped and identified 100% of the isolates in the double blind QA/QC study. (Data from ribotyping QA/QC study can be found in Appendix IV.)

4.2 BST Results

For purposes of data analysis, organization, and presentation, the ribotyped ambient water samples are grouped by source categories. The subjective grouping of ribotypes into source categories merits discussion. The categorization is based to some extent on the basis of biological similarity, but it is also influenced by co-occurrence of species. For example, goat and horse have some biological similarity but even greater similarity from a management viewpoint where they can be grouped into a livestock category that tend to occur on farms and ranches.

One source category is from samples that can not be identified with any known source. This category is referred to as "unknown" source. One cause of "unknowns" is the *E. coli* strains that occur in more than one source type and are, therefore, considered transient strains. For the ribotyping technique used in this study, about 4% of *E. coli* strains would be anticipated to be transient (see section 1.2). Dr. Mansour Samadpour, the principal of IEH, indicated he would anticipate that these transient strains comprise 5 to 10% of the total *E. coli* population found in ambient water samples (Samadpour, 2006). The second cause of "unknowns" is *E. coli* isolates from water samples that do not match any *E. coli* in the known source library. In ribotyping, with the inherent high precision and accuracy of the rRNA methods, data completeness is most affected by the number of ribotypes found that match ribotypes in the known source library. Thus, a large library is important. For this project, in addition to the known source library collected specifically in the Trinity River watershed, the entire IEH library of multiple tens of thousands of library samples were used. But even with the watershed specific library and the large library of IEH, transient strains and unidentified strains are anticipated to comprise 5 to 30% of the *E. coli* in ambient water samples for a typical study (Samadpour, 2006).

Following the ambient water sampling and the known source fecal and sewage ribotype library sampling, the results from each were analyzed and the ribotypes were matched to the project library and to IEH's larger library. Sources were then grouped into six separate categories, which included avian, human, mammalian wildlife, unknown, pet, and livestock. (Results from ribotyping may be found in Appendix V.) Categories were chosen based on similarity of co-occurrence and management practices (Table 4-1).

The avian category included all bird species, wild and domestic that are generally identified under the two sources of waterfowl and avian[2]; the human category included *E. coli* found in raw sewage collected from WWTP influent, WWTP effluent and septage collected from septic service trucks; mammalian wildlife included deer, raccoons, opossums, rabbits, and positive identification of wild canine or feline species, such as coyotes or bobcats; unknown species were those contributing *E. coli* that could not be traced back to any particular source; pets included cats, dogs, and any feline or canine species that could not be definitively identified; and livestock included cows, bison, horses, donkeys, sheep, and pigs. Of note, no large pig or poultry operations were observed in the watershed during library sample collection, and these observations are corroborated by the National Agricultural Statistics data summarized in Table 2-5 that indicate only small numbers of pigs and poultry in the counties associated with the watershed. As will be shown subsequently in the presentation of BST results, pig (or porcine) was not identified in any ambient water samples. The porcine category includes both domestic and feral pigs. Poultry were also not identified as a contributor in any ambient water samples.

During this project, various factors contributed to an unequal number of isolates being ribotyped, on occasion, for the various stations and sampling events. To determine any potential bias during source analysis resulting from the unequal number of isolates, the source data were normalized by calculating the percent source contribution at each station for each sampling event, averaging the results for each station over all events, and finally calculating the overall average percent contribution of all stations per affected segment. The normalized results were then compared to the non-normalized results using the following formula to calculate confidence intervals (CI):

$$\overline{p} \pm z_{\alpha/2}\sqrt{\frac{p(1-p)}{n}}$$

Where p is the estimated proportion of the *E. coli* from a given source, n is the total number of isolates, and $z_{\alpha/2}$ is the value of the standard normal distribution at confidence interval α.

The results from the normalized and non-normalized data analysis for Segments 0806, 0841, and 0805 were found to be very similar and no sources were found to be statistically

[2] More than one avian species can and often do harbor the same *E. coli* strain, which makes unique identification of specific avian species by ribotyping difficult. Waterfowl can, however, often be distinguished from other avian species, and both poultry and Guinea fowl can be characterized. In the present study neither poultry nor Guinea fowl were characterized from any ambient water sample isolates. Though the absence of any contributions from domestic avian species can not be concluded, the estimated livestock populations in Table 2-5 support a presumption that domestic avian species are a minor contributor of bacteria in the West Fork Trinity and Upper Trinity River watersheds.

different at the 95 percent confidence level (Tables 4-2, 4-3, and 4-4). Therefore, due to the simplicity of working with non-normalized data compared to normalized data, the non-normalized data has been used for all remaining analyses.

E. coli results for each of the segments were distributed and analyzed based on overall *E. coli* source distribution, runoff vs. non-runoff events, samples containing *E. coli* concentrations ≤394 cfu/100ml vs. those with >394 cfu/100ml (the single sample criterion), temporal differences between sampling events, and overall *E. coli* source contributions for the individual stations.

Table 4-1. Descriptions of *E. coli* sources as they were categorized based on BST ribotyping results.

Source Category	Source	Description
Avian	Avian	Includes all non-waterfowl avian species; wild and domestic.
	Waterfowl	Includes all waterfowl species.
Human	Sewage	Sewage (Includes all raw sewage from WWTP influent and septage.)
	Wastewater	Wastewater (Includes all treated WWTP effluent.)
Livestock	Bison	
	Bovine	
	Donkey	
	Equine	Includes all equine species that could not be positively identified to the species level.
	Goat	
	Horse	
Mammalian Wildlife	Armadillo	
	Coyote	
	Deer	
	Opossum	
	Rabbit	
	Raccoon	
	Rodent	Includes all rodents that could not be positively identified to the species level.
	Skunk	
	Squirrel	
Pet	Canine	Includes all canine species that could not be positively identified to the species level.
	Cat	
	Dog	
	Feline	Includes all feline species that could not be positively identified to the species level.
Unknown	Unknown	Includes all other species that could not be positively identified.

Table 4-2. Normalized and non-normalized *E. coli* source characterization data for Trinity Segment 0806. (CI = confidence interval)

Segment 0806			Non-Normalized Data			Normalized Data		
Category	**Source**	**Isolate #**	**Contribution (%)**	**95% CI Range**		**Contribution (%)**	**95% CI Range**	
Avian	Avian	81	23.4	15.11	31.71	23.6	15.3	31.9
Avian	Waterfowl	16	4.6	0.51	8.74	4.6	0.5	8.8
Avian	**Subtotal**	**97**	**28.0**	**19.23**	**36.84**	**28.2**	**19.4**	**37.0**
Human	Sewage	2	0.6	-0.91	2.06	0.6	-0.9	2.1
Human	Wastewater	37	10.7	4.64	16.75	10.6	4.5	16.6
Human	**Subtotal**	**39**	**11.3**	**5.07**	**17.47**	**11.2**	**5.0**	**17.3**
Livestock	Bison	0	0.0	0.00	0.00	0.0	0.0	0.0
Livestock	Bovine	5	1.4	-0.89	3.78	1.5	-0.9	3.8
Livestock	Donkey	0	0.0	0.00	0.00	0.0	0.0	0.0
Livestock	Equine	1	0.3	-0.76	1.34	0.3	-0.8	1.4
Livestock	Goat	1	0.3	-0.76	1.34	0.3	-0.8	1.4
Livestock	Horse	6	1.7	-0.82	4.29	1.7	-0.8	4.2
Livestock	**Subtotal**	**13**	**3.8**	**0.03**	**7.48**	**3.8**	**0.0**	**7.5**
Mammalian Wildlife	Armadillo	2	0.6	-0.91	2.06	0.6	-0.9	2.1
Mammalian Wildlife	Coyote	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	Opossum	6	1.7	-0.82	4.29	1.8	-0.8	4.3
Mammalian Wildlife	Rabbit	1	0.3	-0.76	1.34	0.3	-0.8	1.4
Mammalian Wildlife	Raccoon	23	6.6	1.76	11.53	6.7	1.8	11.7
Mammalian Wildlife	Rodent	40	11.6	5.29	17.83	11.1	4.9	17.2
Mammalian Wildlife	Skunk	3	0.9	-0.95	2.68	0.8	-0.9	2.6
Mammalian Wildlife	Squirrel	4	1.2	-0.94	3.25	1.2	-0.9	3.3
Mammalian Wildlife	**Subtotal**	**79**	**22.8**	**14.61**	**31.06**	**22.5**	**14.3**	**30.7**
Pet	Canine	7	2.0	-0.74	4.78	2.0	-0.7	4.8
Pet	Cat	6	1.7	-0.82	4.29	1.8	-0.8	4.3
Pet	Dog	31	9.0	3.36	14.56	9.2	3.5	14.9
Pet	Feline	2	0.6	-0.91	2.06	0.7	-0.9	2.3
Pet	**Subtotal**	**46**	**13.3**	**6.64**	**19.95**	**13.7**	**7.0**	**20.4**
Unknown	Unknown	72	20.8	12.85	28.77	20.7	12.7	28.6
Unknown	**Subtotal**	**72**	**20.8**	**12.85**	**28.77**	**20.7**	**12.7**	**28.6**
	Total	**346**	**100.0**			**100.00**		

Table 4-3. Normalized and non-normalized *E. coli* source characterization data for Trinity Segment 0841. (CI = confidence interval)

Segment 0841			Non-Normalized Data			Normalized Data		
Category	**Source**	**Isolate #**	**Contribution (%)**	**95% CI Range**		**Contribution (%)**	**95% CI Range**	
Avian	Avian	78	22.8	14.58	31.03	22.6	14.4	30.8
Avian	Waterfowl	17	5.0	0.71	9.23	4.9	0.7	9.1
Avian	**Subtotal**	**95**	**27.8**	**19.00**	**36.56**	**27.5**	**18.7**	**36.2**
Human	Sewage	0	0.0	0.00	0.00	0.0	0.0	0.0
Human	Wastewater	70	20.5	12.56	28.38	20.5	12.6	28.5
Human	**Subtotal**	**70**	**20.5**	**12.56**	**28.38**	**20.5**	**12.6**	**28.5**
Livestock	Bison	0	0.0	0.00	0.00	0.0	0.0	0.0
Livestock	Bovine	6	1.8	-0.82	4.33	1.7	-0.8	4.3
Livestock	Donkey	0	0.0	0.00	0.00	0.0	0.0	0.0
Livestock	Equine	3	0.9	-0.95	2.70	0.9	-0.9	2.7
Livestock	Goat	0	0.0	0.00	0.00	0.0	0.0	0.0
Livestock	Horse	4	1.2	-0.94	3.28	1.2	-0.9	3.3
Livestock	**Subtotal**	**13**	**3.8**	**0.05**	**7.55**	**3.8**	**0.0**	**7.5**
Mammalian Wildlife	Armadillo	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	Coyote	2	0.6	-0.91	2.08	0.6	-0.9	2.1
Mammalian Wildlife	Deer	1	0.3	-0.77	1.35	0.3	-0.8	1.4
Mammalian Wildlife	Opossum	3	0.9	-0.95	2.70	0.9	-1.0	2.8
Mammalian Wildlife	Rabbit	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	24	7.0	2.01	12.02	7.0	2.0	12.0
Mammalian Wildlife	Rodent	25	7.3	2.21	12.41	7.3	2.2	12.5
Mammalian Wildlife	Skunk	3	0.9	-0.95	2.70	0.9	-1.0	2.7
Mammalian Wildlife	Squirrel	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**58**	**17.0**	**9.60**	**24.31**	**17.0**	**9.7**	**24.4**
Pet	Canine	5	1.5	-0.89	3.81	1.5	-0.9	3.9
Pet	Cat	4	1.2	-0.94	3.28	1.2	-0.9	3.4
Pet	Dog	34	9.9	4.08	15.81	10.1	4.2	16.0
Pet	Feline	1	0.3	-0.77	1.35	0.3	-0.8	1.3
Pet	**Subtotal**	**44**	**12.9**	**6.30**	**19.43**	**13.0**	**6.4**	**19.7**
Unknown	Unknown	62	18.1	10.58	25.68	18.1	10.6	25.7
Unknown	**Subtotal**	**62**	**18.1**	**10.58**	**25.68**	**18.1**	**10.6**	**25.7**
	Total	**342**	**100.0**			**100.00**		

Table 4-4. Normalized and non-normalized *E. coli* source characterization data for Trinity Segment 0805. (CI = confidence interval)

Segment 0805			Non-Normalized Data			Normalized Data		
Category	**Source**	**Isolate #**	**Contribution (%)**	**95% CI Range**		**Contribution (%)**	**95% CI Range**	
Avian	Avian	99	22.1	14.01	30.29	23.2	15.0	31.5
Avian	Waterfowl	17	3.8	0.05	7.55	3.6	0.0	7.3
Avian	**Subtotal**	**116**	**26.0**	**17.36**	**34.54**	**26.9**	**18.2**	**35.6**
Human	Sewage	0	0.0	0.00	0.00	0.0	0.0	0.0
Human	Wastewater	106	23.7	15.38	32.05	23.5	15.2	31.8
Human	**Subtotal**	**106**	**23.7**	**15.38**	**32.05**	**23.5**	**15.2**	**31.8**
Livestock	Bison	1	0.2	-0.70	1.15	0.2	-0.7	1.2
Livestock	Bovine	29	6.5	1.66	11.32	6.5	1.7	11.3
Livestock	Donkey	3	0.7	-0.93	2.27	0.6	-0.9	2.2
Livestock	Equine	1	0.2	-0.70	1.15	0.2	-0.7	1.2
Livestock	Goat	3	0.7	-0.93	2.27	0.6	-0.9	2.2
Livestock	Horse	12	2.7	-0.48	5.85	2.8	-0.4	6.0
Livestock	**Subtotal**	**49**	**11.0**	**4.84**	**17.09**	**11.0**	**4.9**	**17.1**
Mammalian Wildlife	Armadillo	3	0.7	-0.93	2.27	0.6	-0.9	2.2
Mammalian Wildlife	Coyote	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	Deer	1	0.2	-0.70	1.15	0.2	-0.7	1.2
Mammalian Wildlife	Opossum	2	0.4	-0.86	1.76	0.4	-0.8	1.7
Mammalian Wildlife	Rabbit	1	0.2	-0.70	1.15	0.2	-0.7	1.2
Mammalian Wildlife	Raccoon	27	6.0	1.37	10.71	6.0	1.3	10.6
Mammalian Wildlife	Rodent	29	6.5	1.66	11.32	6.4	1.6	11.2
Mammalian Wildlife	Skunk	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.00	0.00	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**63**	**14.1**	**7.27**	**20.91**	**13.9**	**7.1**	**20.6**
Pet	Canine	6	1.3	-0.91	3.60	1.3	-0.9	3.5
Pet	Cat	9	2.0	-0.74	4.77	2.0	-0.7	4.7
Pet	Dog	36	8.1	2.72	13.39	8.0	2.7	13.3
Pet	Feline	0	0.0	0.00	0.00	0.0	0.0	0.0
Pet	**Subtotal**	**51**	**11.4**	**5.18**	**17.64**	**11.2**	**5.1**	**17.4**
Unknown	Unknown	62	13.9	7.10	20.64	13.5	6.8	20.2
Unknown	**Subtotal**	**62**	**13.9**	**7.10**	**20.64**	**13.5**	**6.8**	**20.2**
	Total	**447**	**100.0**			**100.00**		

During runoff vs. non-runoff analyses, it was determined that a number of sampling events could not be placed into either category due to low rainfall amounts in certain areas or time of sampling relative to the beginning of the storm event. Therefore the isolates were separated and placed into one of the following categories for comparison purposes: 1) definitely runoff influenced, 2) definitely non-runoff influenced, or 3) potentially runoff influenced. The following analyses were then made between the categories: 1) the definite non-runoff isolates vs. the definite runoff isolates, 2) the definite non-runoff isolates vs. the definite runoff isolates (with potential event isolates added), and 3) the definite non-runoff isolates (with potential event isolates added) vs. the definite runoff isolates. Following the comparisons, it was concluded that there was no significant difference (α=0.05) between the three analyses. Thus for brevity of presentation herein the analysis with potential event isolates excluded is provided. This analysis reduces bias by avoiding possibly placing the potentially runoff influence isolates in a category where they may not belong.

4.2.1 Trinity Segment 0806

The West Fork Trinity below Lake Worth (Segment 0806) results were based on 346 isolates collected from stations 18459, 10938, and 16120.

The overall *E. coli* source contribution for Segment 0806 was led by the avian category, with 28.0% of the total contribution (Figure 4-1; Table 4-5). Within the avian category, the major source was found to be the non-waterfowl species, which contributed 23.4% of the total *E. coli* to the affected segment (Table 4-5). Mammalian wildlife was found to be the second highest

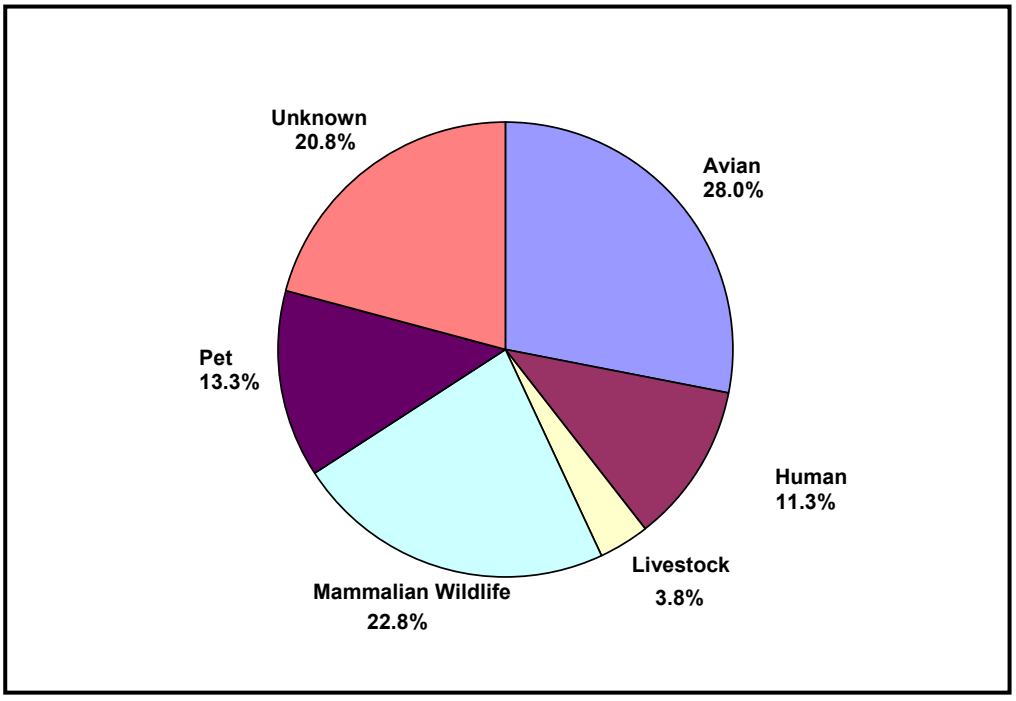

Figure 4-1. *E. coli* source characterization for Trinity Segment 0806 under all conditions.

Table 4-5. Overall *E. coli* source characterization and 95% confidence interval (CI) range for Segment 0806.

Segment 0806					
Category	**Source**	**Isolate #**	**Contribution (%)**	**95% CI Range**	
Avian	Avian	81	23.4	15.11	31.71
Avian	Waterfowl	16	4.6	0.51	8.74
Avian	**Subtotal**	**97**	**28.0**	**19.23**	**36.84**
Human	Sewage	2	0.6	-0.91	2.06
Human	Wastewater	37	10.7	4.64	16.75
Human	**Subtotal**	**39**	**11.3**	**5.07**	**17.47**
Livestock	Bison	0	0	0	0
Livestock	Bovine	5	1.4	-0.89	3.78
Livestock	Donkey	0	0	0	0
Livestock	Equine	1	0.3	-0.76	1.34
Livestock	Goat	1	0.3	-0.76	1.34
Livestock	Horse	6	1.7	-0.82	4.29
Livestock	**Subtotal**	**13**	**3.8**	**0.03**	**7.48**
Mammalian Wildlife	Armadillo	2	0.6	-0.91	2.06
Mammalian Wildlife	Coyote	0	0	0	0
Mammalian Wildlife	Deer	0	0	0	0
Mammalian Wildlife	Opossum	6	1.7	-0.82	4.29
Mammalian Wildlife	Rabbit	1	0.3	-0.76	1.34
Mammalian Wildlife	Raccoon	23	6.6	1.76	11.53
Mammalian Wildlife	Rodent	40	11.6	5.29	17.83
Mammalian Wildlife	Skunk	3	0.9	-0.95	2.68
Mammalian Wildlife	Squirrel	4	1.2	-0.94	3.25
Mammalian Wildlife	**Subtotal**	**79**	**22.8**	**14.61**	**31.06**
Pet	Canine	7	2	-0.74	4.78
Pet	Cat	6	1.7	-0.82	4.29
Pet	Dog	31	9	3.36	14.56
Pet	Feline	2	0.6	-0.91	2.06
Pet	**Subtotal**	**46**	**13.3**	**6.64**	**19.95**
Unknown	Unknown	72	20.8	12.85	28.77
Unknown	**Subtotal**	**72**	**20.8**	**12.85**	**28.77**
	Total	**346**	**100**		

contributor with 22.8%, followed by the unknown category with 20.8%. Within the mammalian wildlife category, the rodent population contributed the most with 11.6%. The top three categories were followed by the pet, human, and livestock categories, respectively. These categories were led by dogs (9.0%) and wastewater (10.7%), while the livestock category's largest sources were horses (1.7%) and bovine (1.4%).

Runoff vs. non-runoff events were not found to be significantly different (α=0.05) based on *E. coli* source contributions (Table 4-6). While not statistically significant, avian contribution was greater for non-runoff events than for runoff events, and both pet and livestock contributions were less for non-runoff events than for runoff events. Segment 0806 had a total of 212 non-runoff influenced isolates and 82 runoff influenced isolates, for a total of 294 isolates. The remaining 52 isolates were collected during events that could not be placed, definitively, in either the non-runoff or runoff categories; therefore they were excluded from the presented analysis.

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Table 4-6. Runoff vs. non-runoff influenced *E. coli* source characterization for Segment 0806. (CI = confidence interval)

Segment 0806		Non-Runoff				Runoff			
Category	Source	Isolate #	Contribution (%)	95% CI Range		Isolate #	Contribution (%)	95% CI Range	
Avian	Avian	51	24.1	15.7	32.4	13	15.9	8.7	23.0
Avian	Waterfowl	11	5.2	0.8	9.5	3	3.7	0.0	7.3
Avian	**Subtotal**	**62**	**29.2**	**20.3**	**38.2**	**16**	**19.5**	**11.7**	**27.3**
Human	Sewage	0	0.0	0.0	0.0	1	1.2	-0.9	3.4
Human	Wastewater	26	12.3	5.8	18.7	9	11.0	4.8	17.1
Human	**Subtotal**	**26**	**12.3**	**5.8**	**18.7**	**10**	**12.2**	**5.8**	**18.6**
Livestock	Bison	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Bovine	5	2.4	-0.6	5.3	0	0.0	0.0	0.0
Livestock	Donkey	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Equine	1	0.5	-0.9	1.8	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0	1	1.2	-0.9	3.4
Livestock	Horse	2	0.9	-1.0	2.8	4	4.9	0.7	9.1
Livestock	**Subtotal**	**8**	**3.8**	**0.0**	**7.5**	**5**	**6.1**	**1.4**	**10.8**
Mammalian Wildlife	Armadillo	2	0.9	-1.0	2.8	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	2	0.9	-1.0	2.8	3	3.7	0.0	7.3
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	11	5.2	0.8	9.5	9	11.0	4.8	17.1
Mammalian Wildlife	Rodent	27	12.7	6.2	19.3	8	9.8	3.9	15.6
Mammalian Wildlife	Skunk	1	0.5	-0.9	1.8	2	2.4	-0.6	5.5
Mammalian Wildlife	Squirrel	4	1.9	-0.8	4.6	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**47**	**22.2**	**14.0**	**30.3**	**22**	**26.8**	**18.1**	**35.5**
Pet	Canine	4	1.9	-0.8	4.6	3	3.7	0.0	7.3
Pet	Cat	2	0.9	-1.0	2.8	4	4.9	0.7	9.1
Pet	Dog	20	9.4	3.7	15.2	7	8.5	3.1	14.0
Pet	Feline	1	0.5	-0.9	1.8	1	1.2	-0.9	3.4
Pet	**Subtotal**	**27**	**12.7**	**6.2**	**19.3**	**15**	**18.3**	**10.7**	**25.9**
Unknown	Unknown	42	19.8	12.0	27.6	14	17.1	9.7	24.4
Unknown	**Subtotal**	**42**	**19.8**	**12.0**	**27.6**	**14**	**17.1**	**9.7**	**24.4**
	Total	**212**	**100**			**82**	**100**		

E. coli source contributions for samples containing ≤394 cfu/100ml were not found to be significantly different (α=0.05) from samples containing >394 cfu/100 ml (Table 4-7).

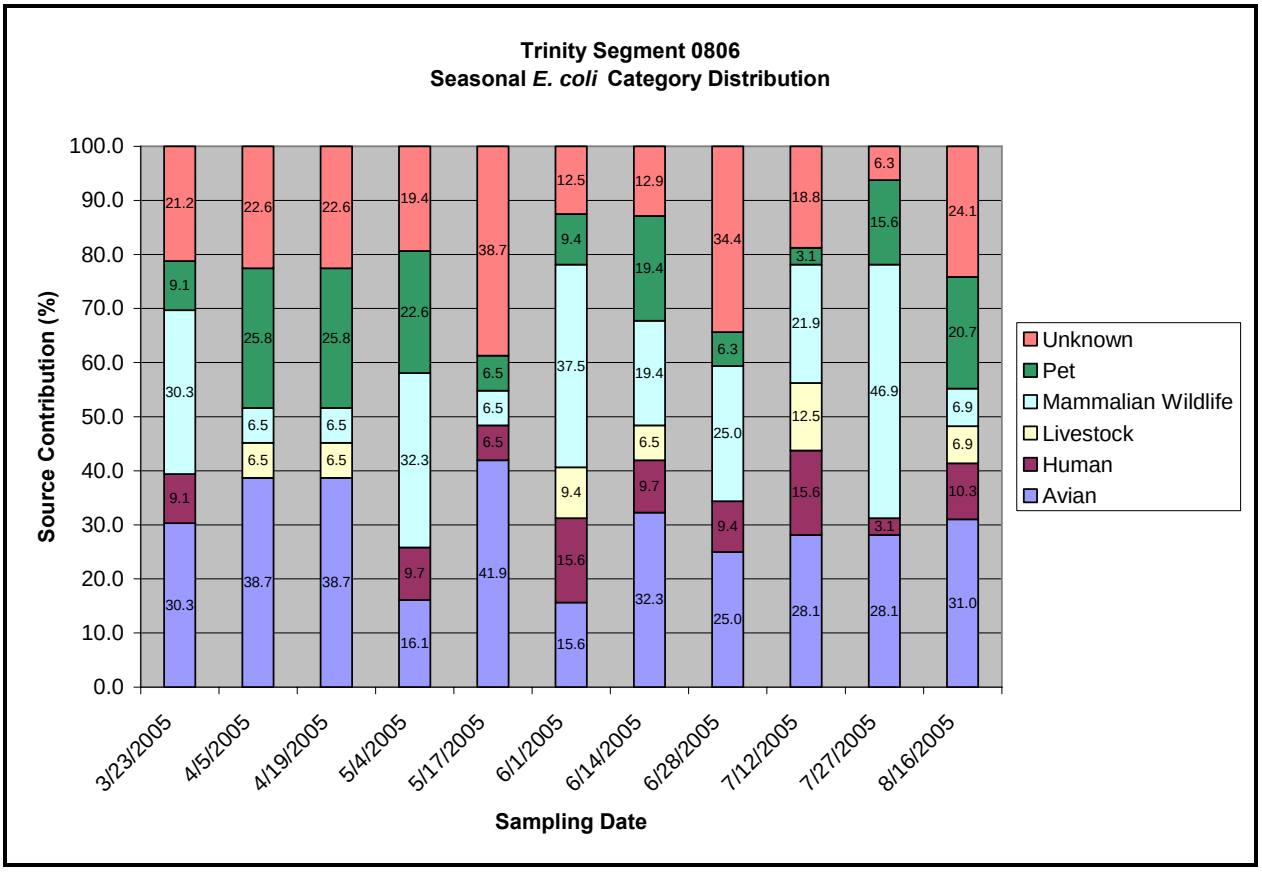
Table 4-7. *E. coli* source characterization for samples containing ≤394 cfu/100 ml vs. samples containing >394 cfu/100ml for Segment 0806. (CI = confidence interval)

Segment 0806		Samples ≤ 394 cfu / 100ml				Samples > 394 cfu / 100ml			
Category	Source	Isolate #	Contribution (%)	95% CI Range		Isolate #	Contribution (%)	95% CI Range	
Avian	Avian	67	26.0	17.4	34.6	14	15.9	8.7	23.1
Avian	Waterfowl	13	5.0	0.8	9.3	3	3.4	-0.1	7.0
Avian	Subtotal	80	31.0	21.9	40.1	17	19.3	11.6	27.1
Human	Sewage	1	0.4	-0.8	1.6	1	1.1	-0.9	3.2
Human	Wastewater	28	10.9	4.8	16.9	9	10.2	4.3	16.2
Human	Subtotal	29	11.2	5.0	17.4	10	11.4	5.1	17.6
Livestock	Bison	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Bovine	5	1.9	-0.8	4.6	0	0.0	0.0	0.0
Livestock	Donkey	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Equine	0	0.0	0.0	0.0	1	1.1	-0.9	3.2
Livestock	Goat	0	0.0	0.0	0.0	1	1.1	-0.9	3.2
Livestock	Horse	2	0.8	-0.9	2.5	4	4.5	0.5	8.6
Livestock	Subtotal	7	2.7	-0.5	5.9	6	6.8	1.9	11.8
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0	2	2.3	-0.6	5.2
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	3	1.2	-0.9	3.3	3	3.4	-0.1	7.0
Mammalian Wildlife	Rabbit	1	0.4	-0.8	1.6	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	14	5.4	1.0	9.9	9	10.2	4.3	16.2
Mammalian Wildlife	Rodent	31	12.0	5.6	18.4	9	10.2	4.3	16.2
Mammalian Wildlife	Skunk	1	0.4	-0.8	1.6	2	2.3	-0.6	5.2
Mammalian Wildlife	Squirrel	4	1.6	-0.9	4.0	0	0.0	0.0	0.0
Mammalian Wildlife	Subtotal	54	20.9	13.0	28.9	25	28.4	19.6	37.2
Pet	Canine	4	1.6	-0.9	4.0	3	3.4	-0.1	7.0
Pet	Cat	1	0.4	-0.8	1.6	5	5.7	1.1	10.2
Pet	Dog	24	9.3	3.6	15.0	7	8.0	2.7	13.3
Pet	Feline	1	0.4	-0.8	1.6	1	1.1	-0.9	3.2
Pet	Subtotal	30	11.6	5.3	17.9	16	18.2	10.6	25.7
Unknown	Unknown	58	22.5	14.3	30.7	14	15.9	8.7	23.1
Unknown	Subtotal	58	22.5	14.3	30.7	14	15.9	8.7	23.1
	Total	**258**	**100.0**			**88**	**100.0**		

Largely similar to the comparisons of runoff and non-runoff events, avian contribution was greater for *E. coli* isolates from samples containing ≤394 cfu/100 ml than for samples containing >394 cfu/100 ml, and mammalian wildlife, pet, and livestock contribution were less for samples containing ≤394 cfu/100 ml than for samples containing >394 cfu/100 ml. But as with runoff vs. non-runoff analysis, the differences were not statistically significant for α=0.05. Segment 0806 had a total of 258 *E. coli* isolates from samples with ≤394 cfu/100 ml and 88 isolates from samples with >394 cfu/100ml, for a total of 346 isolates.

Temporal contributions of *E. coli* isolates by the various categories were analyzed and graphed in order to identify differences between sampling events and possibly determine whether or not there were temporal patterns present (Figure 4-2.). The categorical contributions results for Segment 0806 did not appear to contain any strong seasonal patterns and variations appeared to be more random than systematically associated with time.

Figure 4-2. Segment 0806 temporal *E. coli* category distribution.



4.2.1.1 Station 18459- Northside Drive (Tarrant County)

Station 18459 results were based on 116 isolates. The highest contributor of *E. coli* contamination at the uppermost station, 18459, was found to be avian in nature with a 25.0% contribution (Figure 4-3; Table 4-8). Mammalian wildlife was the next highest contributor at 23.3%, followed by the unknown category with 19.8%. The human, pet, and livestock categories made up the remaining 32%. The source results for station 18459 were found to be similar to the overall segment distribution. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

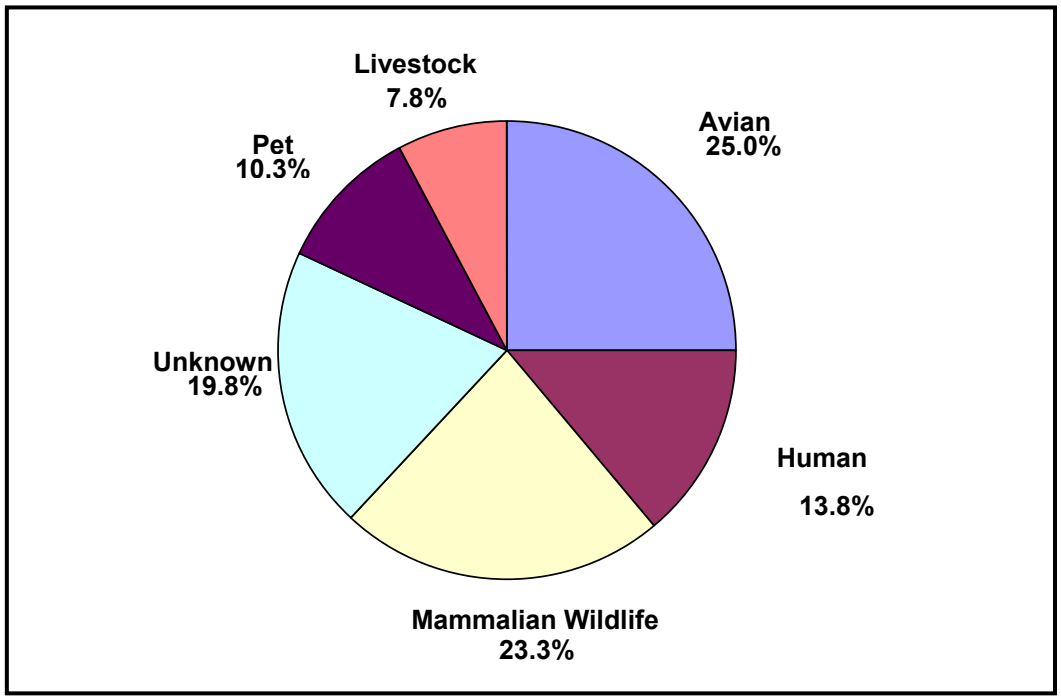


Figure 4-3. *E. coli* source characterization for station 18459 under all conditions.

Table 4-8. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 18459.

Station 18459					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	22	19.0	11.3	26.6
Avian	Waterfowl	7	6.0	1.4	10.7
Avian	**Subtotal**	**29**	**25.0**	**16.5**	**33.5**
Human	Sewage	2	1.7	-0.8	4.3
Human	Wastewater	14	12.1	5.7	18.5
Human	**Subtotal**	**16**	**13.8**	**7.0**	**20.6**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	4	3.4	-0.1	7.0
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	1	0.9	-0.9	2.7
Livestock	Goat	1	0.9	-0.9	2.7
Livestock	Horse	3	2.6	-0.5	5.7
Livestock	**Subtotal**	**9**	**7.8**	**2.5**	**13.0**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	1	0.9	-0.9	2.7
Mammalian Wildlife	Raccoon	8	6.9	1.9	11.9
Mammalian Wildlife	Rodent	15	12.9	6.4	19.5
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	3	2.6	-0.5	5.7
Mammalian Wildlife	**Subtotal**	**27**	**23.3**	**15.0**	**31.6**
Pet	Canine	4	3.4	-0.1	7.0
Pet	Cat	0	0.0	0.0	0.0
Pet	Dog	8	6.9	1.9	11.9
Pet	Feline	0	0.0	0.0	0.0
Pet	**Subtotal**	**12**	**10.3**	**4.4**	**16.3**
Unknown	Unknown	23	19.8	12.0	27.6
Unknown	**Subtotal**	**23**	**19.8**	**12.0**	**27.6**
	Total	**116**	**100.0**		

4.2.1.2 Station 10938- Beach Street (Tarrant County)

Station 10938 results were based on 116 isolates. The highest contributors of *E. coli* contamination at station 10938 were the avian and unknown categories, both with 26.7% apiece (Figure 4-4; Table 4-9). Mammalian wildlife was next, representing 18.1% of the contribution. Following wildlife, the pet population was fourth in total contribution, with approximately 16% of the total. The human and livestock populations represented the lowest contributors, with 12.1% and 0.9%, respectively. Compared to the overall segment distribution, the results for station 10938 were again found to be similar. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

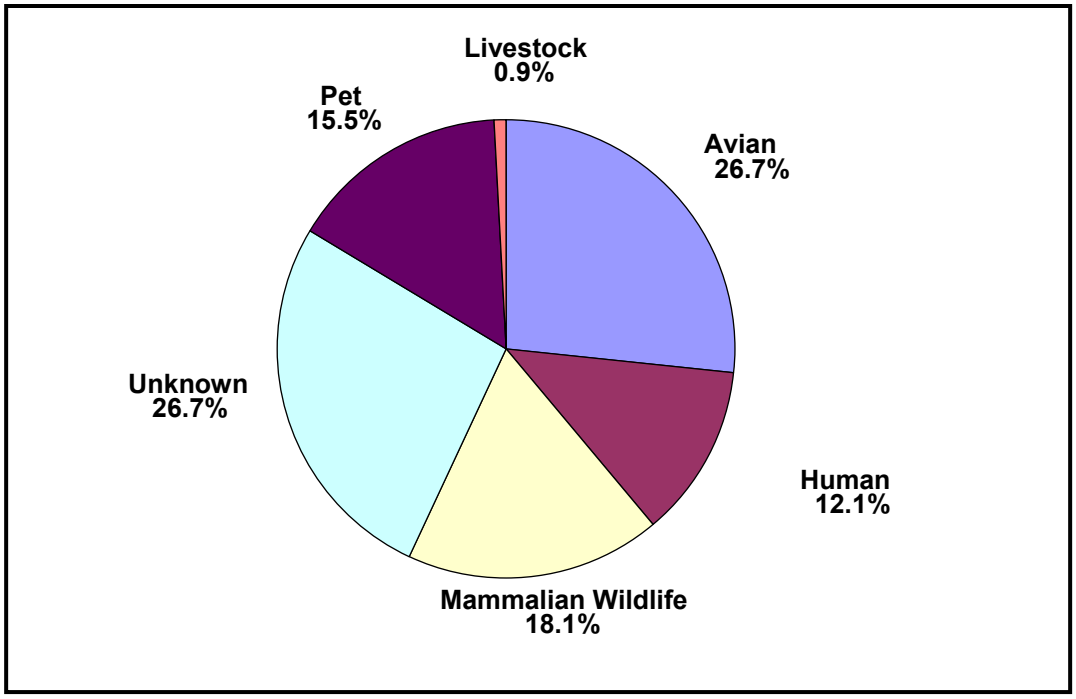


Figure 4-4. *E. coli* source characterization for station 10938 under all conditions.

Table 4-9. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 10938.

Station 10938					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	28	24.1	15.8	32.5
Avian	Waterfowl	3	2.6	-0.5	5.7
Avian	**Subtotal**	**31**	**26.7**	**18.1**	**35.4**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	14	12.1	5.7	18.5
Human	**Subtotal**	**14**	**12.1**	**5.7**	**18.5**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	1	0.9	-0.9	2.7
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	0	0.0	0.0	0.0
Livestock	**Subtotal**	**1**	**0.9**	**-0.9**	**2.7**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	2	1.7	-0.8	4.3
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	8	6.9	1.9	11.9
Mammalian Wildlife	Rodent	10	8.6	3.1	14.1
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	1	0.9	-0.9	2.7
Mammalian Wildlife	**Subtotal**	**21**	**18.1**	**10.6**	**25.7**
Pet	Canine	2	1.7	-0.8	4.3
Pet	Cat	2	1.7	-0.8	4.3
Pet	Dog	13	11.2	5.0	17.4
Pet	Feline	1	0.9	-0.9	2.7
Pet	**Subtotal**	**18**	**15.5**	**8.4**	**22.6**
Unknown	Unknown	31	26.7	18.1	35.4
Unknown	**Subtotal**	**31**	**26.7**	**18.1**	**35.4**
	Total	**116**	**100.0**		

4.2.1.3 Station 16120- Handley-Ederville (Tarrant County)

Station 16120 results were based on 114 isolates. The highest contributor of *E. coli* contamination at station 16120 was the avian category, representing 32.5 % of the total (Figure 4-5; Table 4-10). The non-waterfowl species contributed 27.2% of the total station concentration, equaling the mammalian wildlife, the second highest category. This category was also once again led by the rodent population, with a little over 13% of the total. The top two categorical contributors were followed by the unknown category with 15.8%. The pet, human, and livestock contributions, respectively, were the lowest contributors of *E. coli* at station 16120. Compared to the overall segment distribution, the results for station 16120 were very similar to the overall segment distribution. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

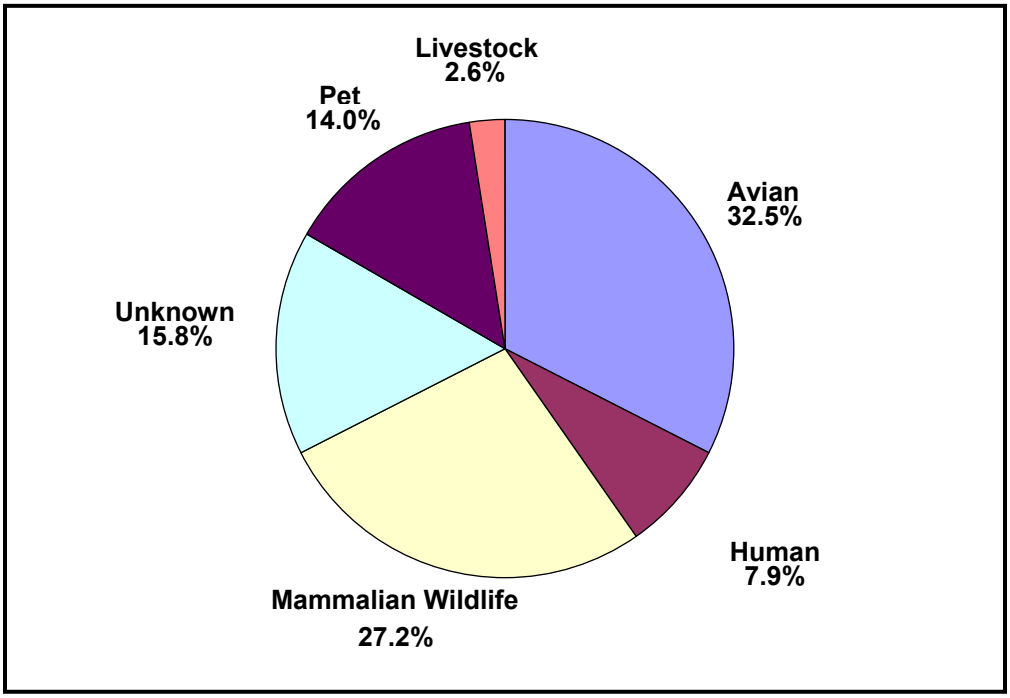


Figure 4-5. *E. coli* source characterization for station 16120 under all conditions.

Table 4-10. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 16120.

Station 16120					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	31	27.2	18.5	35.9
Avian	Waterfowl	6	5.3	0.9	9.6
Avian	**Subtotal**	**37**	**32.5**	**23.3**	**41.6**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	9	7.9	2.6	13.2
Human	**Subtotal**	**9**	**7.9**	**2.6**	**13.2**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	0	0.0	0.0	0.0
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	3	2.6	-0.5	5.8
Livestock	**Subtotal**	**3**	**2.6**	**-0.5**	**5.8**
Mammalian Wildlife	Armadillo	2	1.8	-0.8	4.3
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	4	3.5	-0.1	7.1
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	7	6.1	1.4	10.8
Mammalian Wildlife	Rodent	15	13.2	6.5	19.8
Mammalian Wildlife	Skunk	3	2.6	-0.5	5.8
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**31**	**27.2**	**18.5**	**35.9**
Pet	Canine	1	0.9	-1.0	2.7
Pet	Cat	4	3.5	-0.1	7.1
Pet	Dog	10	8.8	3.2	14.3
Pet	Feline	1	0.9	-1.0	2.7
Pet	**Subtotal**	**16**	**14.0**	**7.2**	**20.8**
Unknown	Unknown	18	15.8	8.6	22.9
Unknown	**Subtotal**	**18**	**15.8**	**8.6**	**22.9**
	Total	**114**	**100.0**		

4.2.2 Trinity Segment 0841

The Lower West Fork Trinity River (Segment 0841) results were based on 342 isolates collected from stations 17669, 11081, and 11089. The overall *E. coli* source contribution for Segment 0841 was led by the avian category, representing 27.8% of the total, followed by the human contribution, which made up 20.5% (Figure 4-6; Table 4-11). The unknown category made up 18.1% of the total segment contribution, followed closely by mammalian wildlife. Within the wildlife category rodent and raccoon were the dominant contributors. The pet and livestock categories were found to be the lowest contributors of *E. coli* in this particular segment. Overall, sources of *E. coli* in Segment 0841 were found to be essentially the same as the results from upstream Segment 0806.

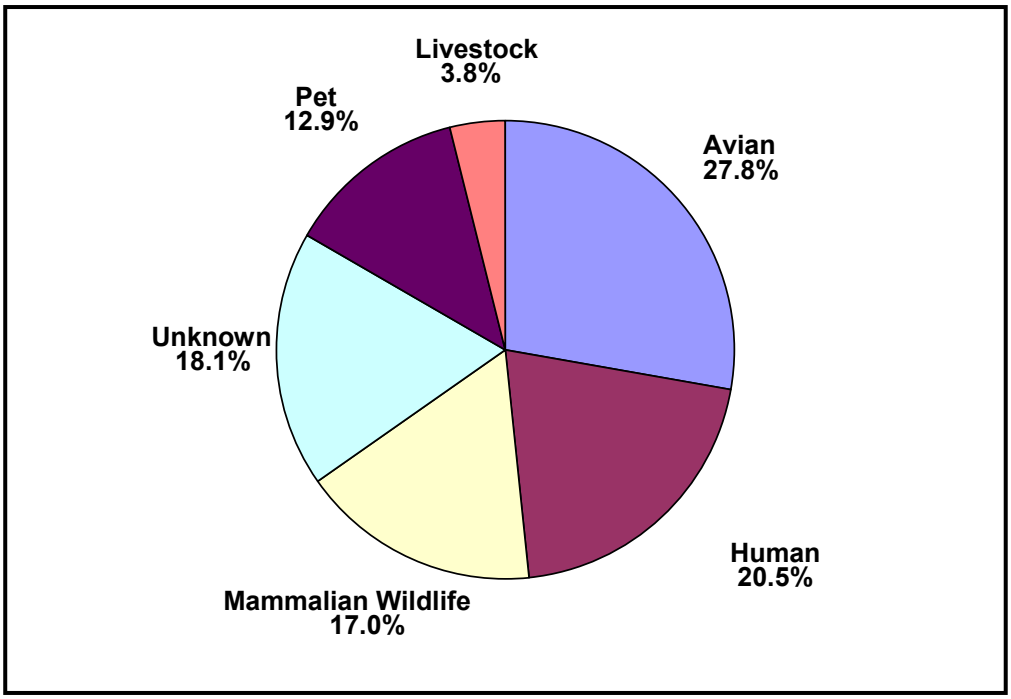


Figure 4-6. *E. coli* source characterization for Trinity Segment 0841 under all conditions.

Runoff vs. non-runoff source contributions were not found to be significantly different (α=0.05) based on *E. coli* source contributions (Table 4-12). As found in Segment 0806, some differences in source contributions that are not statistically significant can be observed in the data. Avian and pet contributions increased from non-runoff to runoff events, and mammalian wildlife and the unknown category decreased from non-runoff to runoff events. Segment 0841had a total of 187 non-runoff influenced isolates and 102 runoff influenced isolates, for a total of 289 isolates. The remaining 53 isolates were collected during events that could not be, definitively, placed in either the non-runoff or runoff categories. Therefore, as stated earlier in the results background information, the indefinite isolates were excluded from this particular analysis.

Table 4-11. Overall *E. coli* source characterization and 95% confidence interval (CI) range for Segment 0841.

Segment 0841					
Category	**Source**	**Isolate #**	**Contribution (%)**	**95% CI Range**	
Avian	Avian	78	22.8	14.58	31.03
Avian	Waterfowl	17	5.0	0.71	9.23
Avian	**Subtotal**	**95**	**27.8**	**19.00**	**36.56**
Human	Sewage	0	0.0	0.00	0.00
Human	Wastewater	70	20.5	12.56	28.38
Human	**Subtotal**	**70**	**20.5**	**12.56**	**28.38**
Livestock	Bison	0	0.0	0.00	0.00
Livestock	Bovine	6	1.8	-0.82	4.33
Livestock	Donkey	0	0.0	0.00	0.00
Livestock	Equine	3	0.9	-0.95	2.70
Livestock	Goat	0	0.0	0.00	0.00
Livestock	Horse	4	1.2	-0.94	3.28
Livestock	**Subtotal**	**13**	**3.8**	**0.05**	**7.55**
Mammalian Wildlife	Armadillo	0	0.0	0.00	0.00
Mammalian Wildlife	Coyote	2	0.6	-0.91	2.08
Mammalian Wildlife	Deer	1	0.3	-0.77	1.35
Mammalian Wildlife	Opossum	3	0.9	-0.95	2.70
Mammalian Wildlife	Rabbit	0	0.0	0.00	0.00
Mammalian Wildlife	Raccoon	24	7.0	2.01	12.02
Mammalian Wildlife	Rodent	25	7.3	2.21	12.41
Mammalian Wildlife	Skunk	3	0.9	-0.95	2.70
Mammalian Wildlife	Squirrel	0	0.0	0.00	0.00
Mammalian Wildlife	**Subtotal**	**58**	**17.0**	**9.60**	**24.31**
Pet	Canine	5	1.5	-0.89	3.81
Pet	Cat	4	1.2	-0.94	3.28
Pet	Dog	34	9.9	4.08	15.81
Pet	Feline	1	0.3	-0.77	1.35
Pet	**Subtotal**	**44**	**12.9**	**6.30**	**19.43**
Unknown	Unknown	62	18.1	10.58	25.68
Unknown	**Subtotal**	**62**	**18.1**	**10.58**	**25.68**
	Total	**342**	**100.0**		

Table 4-12. Runoff vs. non-runoff influenced *E. coli* source characterization for Trinity Segment 0841. (CI = confidence interval)

Segment 0841		Non-Runoff				Runoff			
Category	Source	Isolate #	Contribution (%)	95% CI Range		Isolate #	Contribution (%)	95% CI Range	
Avian	Avian	37	19.8	12.0	27.6	28	27.5	18.7	36.2
Avian	Waterfowl	9	4.8	0.6	9.0	5	4.9	0.7	9.1
Avian	**Subtotal**	**46**	**24.6**	**16.2**	**33.0**	**33**	**32.4**	**23.2**	**41.5**
Human	Sewage	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Human	Wastewater	40	21.4	13.4	29.4	20	19.6	11.8	27.4
Human	**Subtotal**	**40**	**21.4**	**13.4**	**29.4**	**20**	**19.6**	**11.8**	**27.4**
Livestock	Bison	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Bovine	2	1.1	-0.9	3.1	4	3.9	0.1	7.7
Livestock	Donkey	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Equine	3	1.6	-0.9	4.1	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Horse	3	1.6	-0.9	4.1	1	1.0	-1.0	2.9
Livestock	**Subtotal**	**8**	**4.3**	**0.3**	**8.2**	**5**	**4.9**	**0.7**	**9.1**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	2	1.1	-0.9	3.1	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0	1	1.0	-1.0	2.9
Mammalian Wildlife	Opossum	1	0.5	-0.9	2.0	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	15	8.0	2.7	13.3	4	3.9	0.1	7.7
Mammalian Wildlife	Rodent	17	9.1	3.5	14.7	7	6.9	1.9	11.8
Mammalian Wildlife	Skunk	2	1.1	-0.9	3.1	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**37**	**19.8**	**12.0**	**27.6**	**12**	**11.8**	**5.4**	**18.1**
Pet	Canine	2	1.1	-0.9	3.1	2	2.0	-0.8	4.7
Pet	Cat	1	0.5	-0.9	2.0	2	2.0	-0.8	4.7
Pet	Dog	17	9.1	3.5	14.7	13	12.7	6.2	19.3
Pet	Feline	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Pet	**Subtotal**	**20**	**10.7**	**4.6**	**16.8**	**17**	**16.7**	**9.4**	**24.0**
Unknown	Unknown	36	19.3	11.5	27.0	15	14.7	7.8	21.6
Unknown	**Subtotal**	**36**	**19.3**	**11.5**	**27.0**	**15**	**14.7**	**7.8**	**21.6**
	Total	**187**	**100**			**102**	**100**		

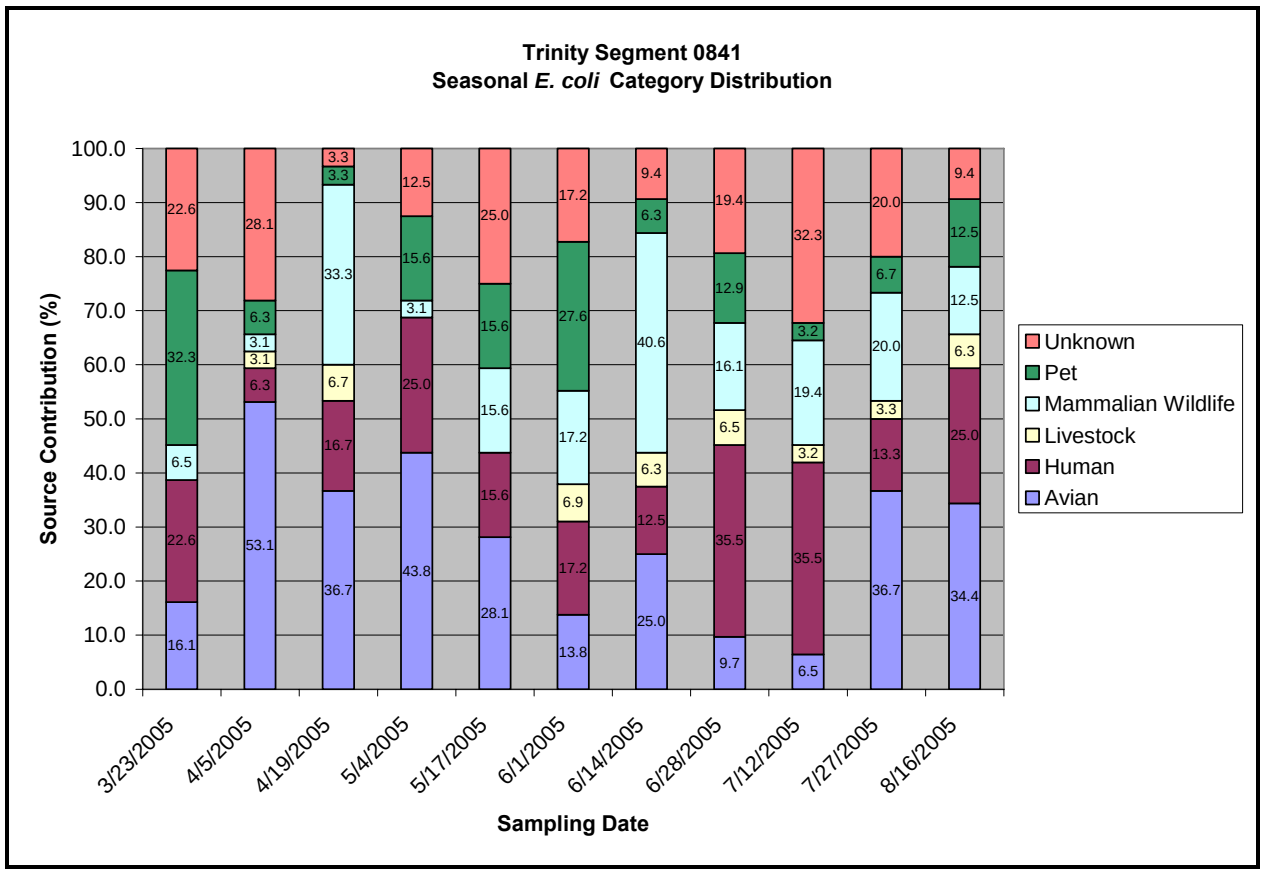
E. coli source contributions for samples containing ≤394 cfu/100ml were not found to be significantly different (α=0.05) from samples containing >394 cfu/100ml (Table 4-13). Again, some non-statistically significant differences may be observed. Avian contributions are higher for the samples containing >394 cfu/100ml, while mammalian wildlife and unknown source contributions are higher for samples containing ≤394 cfu/100ml. Segment 0841 had a total of 225 isolates from samples with ≤394 cfu/100ml and 117 isolates from samples with >394 cfu/100ml, for a total of 342 isolates.

Table 4-13. *E. coli* source characterization for samples containing ≤394 cfu/100ml vs. those with >394 cfu/100ml for Segment 0841. (CI = confidence interval)

Segment 0841		Samples ≤ 394 cfu / 100ml				Samples > 394 cfu / 100ml			
Category	Source	Isolate #	Contribution (%)	95% CI Range		Isolate #	Contribution (%)	95% CI Range	
Avian	Avian	45	20.0	12.2	27.8	33	28.2	19.4	37.0
Avian	Waterfowl	11	4.9	0.7	9.1	6	5.1	0.8	9.5
Avian	**Subtotal**	**56**	**24.9**	**16.4**	**33.4**	**39**	**33.3**	**24.1**	**42.6**
Human	Sewage	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Human	Wastewater	47	20.9	12.9	28.9	23	19.7	11.9	27.4
Human	**Subtotal**	**47**	**20.9**	**12.9**	**28.9**	**23**	**19.7**	**11.9**	**27.4**
Livestock	Bison	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Bovine	2	0.9	-1.0	2.7	4	3.4	-0.1	7.0
Livestock	Donkey	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Equine	3	1.3	-0.9	3.6	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Livestock	Horse	3	1.3	-0.9	3.6	1	0.9	-0.9	2.7
Livestock	**Subtotal**	**8**	**3.6**	**-0.1**	**7.2**	**5**	**4.3**	**0.3**	**8.2**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	2	0.9	-1.0	2.7	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0	1	0.9	-0.9	2.7
Mammalian Wildlife	Opossum	3	1.3	-0.9	3.6	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	17	7.6	2.4	12.7	7	6.0	1.3	10.6
Mammalian Wildlife	Rodent	18	8.0	2.7	13.3	7	6.0	1.3	10.6
Mammalian Wildlife	Skunk	3	1.3	-0.9	3.6	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**43**	**19.1**	**11.4**	**26.8**	**15**	**12.8**	**6.3**	**19.4**
Pet	Canine	3	1.3	-0.9	3.6	2	1.7	-0.8	4.2
Pet	Cat	2	0.9	-1.0	2.7	2	1.7	-0.8	4.2
Pet	Dog	22	9.8	4.0	15.6	12	10.3	4.3	16.2
Pet	Feline	0	0.0	0.0	0.0	1	0.9	-0.9	2.7
Pet	**Subtotal**	**27**	**12.0**	**5.6**	**18.4**	**17**	**14.5**	**7.6**	**21.4**
Unknown	Unknown	44	19.6	11.8	27.3	18	15.4	8.3	22.5
Unknown	**Subtotal**	**44**	**19.6**	**11.8**	**27.3**	**18**	**15.4**	**8.3**	**22.5**
	Total	**225**	**100.0**			**117**	**100.0**		

Temporal contributions of *E. coli* isolates by the various categories were analyzed and graphed in order to identify differences between sampling events and possibly determine whether or not there were temporal patterns present (Figure 4-7). The categorical contributions for Segment 0841 did not exhibit any strong seasonal patterns, and variations appear to be more random than systematically associated with time.

Figure 4-7. Segment 0841 temporal *E. coli* category distribution.



4.2.2.1 Station 17669- Roy Orr (Dallas County)

Station 17669 results were based on 113 isolates. The largest contributor of *E. coli* at station 17669 was due to avian influence, which made up 28.3% of the total (Figure 4-8; Table 4-14). Human influence was next with 19.5%, while mammalian wildlife contributed 18.6%.At station 17669, the raccoon contribution, 8.0%, surpassed the rodent contribution, which contributed 7.1%. The unknown category was the fourth highest contributor, followed by pets and livestock, respectively. The pet category was once again led by the dogs with 10.6%, and the livestock category was found to be co-dominated again by bovine and equine species. The results for station 17669 were found to be very similar to the overall segment findings. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

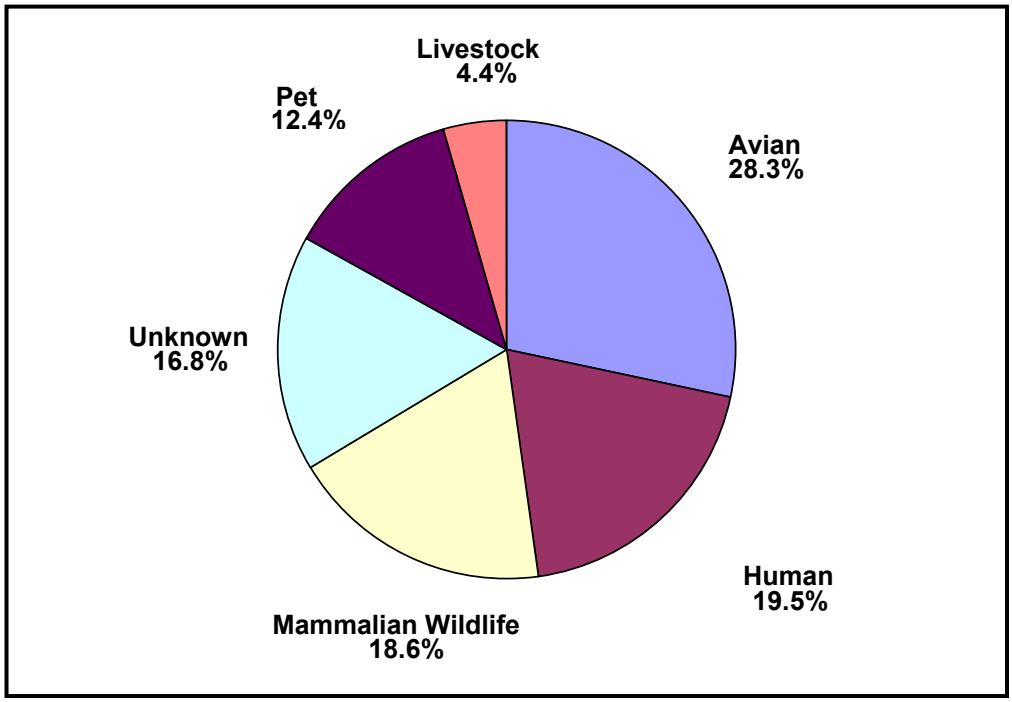


Figure 4-8. *E. coli* source characterization for station 17669 under all conditions.

Table 4-14. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 17669.

Station 17669					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	25	22.1	14.0	30.3
Avian	Waterfowl	7	6.2	1.5	10.9
Avian	**Subtotal**	**32**	**28.3**	**19.5**	**37.1**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	22	19.5	11.7	27.2
Human	**Subtotal**	**22**	**19.5**	**11.7**	**27.2**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	2	1.8	-0.8	4.4
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	2	1.8	-0.8	4.4
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	1	0.9	-1.0	2.7
Livestock	**Subtotal**	**5**	**4.4**	**0.4**	**8.5**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	1	0.9	-1.0	2.7
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	1	0.9	-1.0	2.7
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	9	8.0	2.7	13.3
Mammalian Wildlife	Rodent	8	7.1	2.1	12.1
Mammalian Wildlife	Skunk	2	1.8	-0.8	4.4
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**21**	**18.6**	**11.0**	**26.2**
Pet	Canine	0	0.0	0.0	0.0
Pet	Cat	1	0.9	-1.0	2.7
Pet	Dog	12	10.6	4.6	16.7
Pet	Feline	1	0.9	-1.0	2.7
Pet	**Subtotal**	**14**	**12.4**	**5.9**	**18.8**
Unknown	Unknown	19	16.8	9.5	24.1
Unknown	**Subtotal**	**19**	**16.8**	**9.5**	**24.1**
	Total	**113**	**100.0**		

4.2.2.2 Station 11081- Belt Line Road (Dallas County)

Station 11081 results were based on 115 isolates. At station 11081, avian population was once again the highest contributor of *E. coli* bacteria, representing 31.3% of the total contribution (Figure 4-9; Table 4-15). The next highest source of contamination came from the unknown category with 20.0%. Human addition to the concentration of *E. coli* was third highest with 18.3%. The mammalian wildlife, pet, and livestock contributions represented the three lowest contributors of *E. coli* at this particular station. There were no substantial differences between the overall segment distribution and station 11081. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

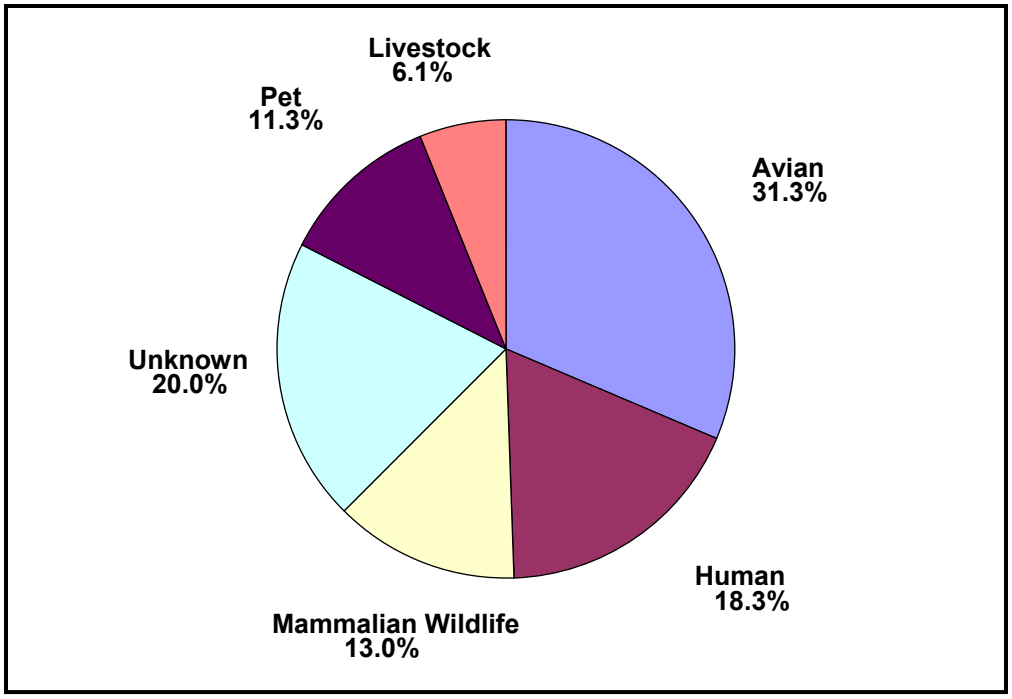


Figure 4-9. *E. coli* source characterization for station 11081 under all conditions.

Table 4-15. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 11081.

Station 11081					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	30	26.1	17.5	34.7
Avian	Waterfowl	6	5.2	0.9	9.6
Avian	**Subtotal**	**36**	**31.3**	**22.2**	**40.4**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	21	18.3	10.7	25.8
Human	**Subtotal**	**21**	**18.3**	**10.7**	**25.8**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	4	3.5	-0.1	7.1
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	3	2.6	-0.5	5.7
Livestock	**Subtotal**	**7**	**6.1**	**1.4**	**10.8**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	1	0.9	-1.0	2.7
Mammalian Wildlife	Deer	1	0.9	-1.0	2.7
Mammalian Wildlife	Opossum	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	6	5.2	0.9	9.6
Mammalian Wildlife	Rodent	7	6.1	1.4	10.8
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**15**	**13.0**	**6.4**	**19.6**
Pet	Canine	2	1.7	-0.8	4.3
Pet	Cat	3	2.6	-0.5	5.7
Pet	Dog	8	7.0	2.0	11.9
Pet	Feline	0	0.0	0.0	0.0
Pet	**Subtotal**	**13**	**11.3**	**5.1**	**17.5**
Unknown	Unknown	23	20.0	12.2	27.8
Unknown	**Subtotal**	**23**	**20.0**	**12.2**	**27.8**
	Total	**115**	**100.0**		

4.2.2.3 Station 11089- West Loop 12 (Dallas County)

Station 11089 results were based on 114 isolates. Avian and human populations shared the largest contribution of *E. coli* at station 11089 with 23.7% each, while the mammalian wildlife contribution represented 19.3% (Figure 4-10; Table 4-16). The unknown, pet, and livestock populations, respectively, represented the lowest contributors. Compared to the overall segment, the results for this station were found to be similar. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

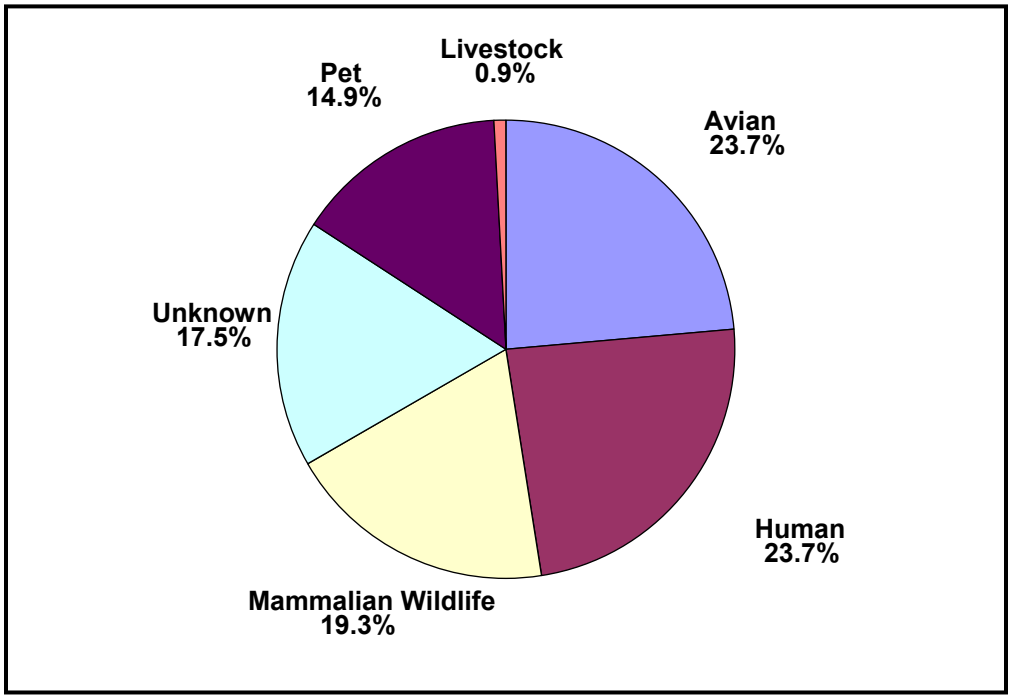


Figure 4-10. *E. coli* source characterization for station 11089 under all conditions.

Table 4-16. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 11089.

Station 11089					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	23	20.2	12.3	28.0
Avian	Waterfowl	4	3.5	-0.1	7.1
Avian	**Subtotal**	**27**	**23.7**	**15.4**	**32.0**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	27	23.7	15.4	32.0
Human	**Subtotal**	**27**	**23.7**	**15.4**	**32.0**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	0	0.0	0.0	0.0
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	1	0.9	-1.0	2.7
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	0	0.0	0.0	0.0
Livestock	**Subtotal**	**1**	**0.9**	**-1.0**	**2.7**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	2	1.8	-0.8	4.3
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	9	7.9	2.6	13.2
Mammalian Wildlife	Rodent	10	8.8	3.2	14.3
Mammalian Wildlife	Skunk	1	0.9	-1.0	2.7
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**22**	**19.3**	**11.6**	**27.0**
Pet	Canine	3	2.6	-0.5	5.8
Pet	Cat	0	0.0	0.0	0.0
Pet	Dog	14	12.3	5.8	18.7
Pet	Feline	0	0.0	0.0	0.0
Pet	**Subtotal**	**17**	**14.9**	**7.9**	**21.9**
Unknown	Unknown	20	17.5	10.1	25.0
Unknown	**Subtotal**	**20**	**17.5**	**10.1**	**25.0**
	Total	**114**	**100.0**		

4.2.3 Trinity Segment 0805

The Upper Trinity River (Segment 0805) results were based on 447 isolates collected from stations 10937, 10934, 10925, and 10924. Overall *E. coli* source contributions for Segment 0805 was led by avian species with 26.0% of the total (Figure 4-11; Table 4-17). Following avian species, the human category was found to contribute 23.7%, while mammalian wildlife contributed 14.3%. The unknown, pet, and livestock species contributions were the lowest in Segment 0805. Because of the length of Segment 0805 (100 miles) and the upstream to downstream transition from urban to rural land use/land cover, only within this segment was there found to be a statistically significant spatial variability in source contributions. Most notably, livestock contributions are significantly greater for the two downstream stations (10925 and 10924) than the two upstream stations (10937 and 10934), while other categories show much smaller, statistically non-significant changes. (The differences in livestock contribution are provided in more detail under individual station discussions.) Overall, sources of *E. coli* in Segment 0805 were found to be essentially the same as the results from upstream Segments 0806 and 0841.

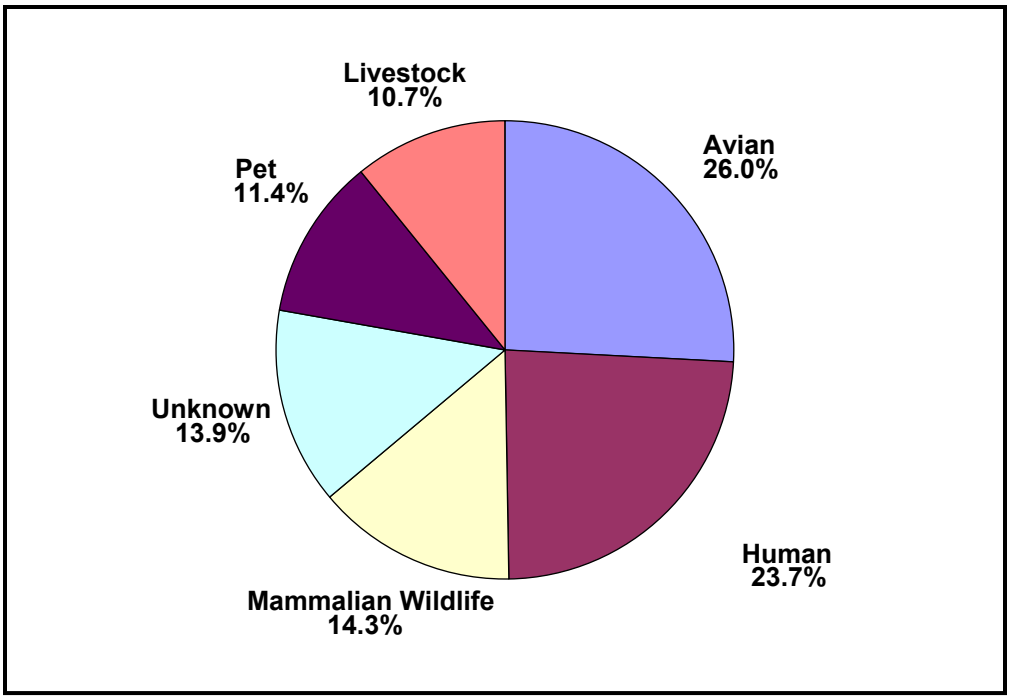


Figure 4-11. *E. coli* source characterization for Segment 0805 under all conditions.

Runoff vs. non-runoff events were not found to be significantly different (α=0.05) based on *E. coli* source contributions (Table 4-18). While not statistically significant, mammalian wildlife contribution appeared to decrease from non-runoff to runoff events, while pet contribution increased. Segment 0805 had a total of 282 non-runoff influenced isolates and 102 runoff influenced isolates for a total of 384 isolates. The remaining 63 isolates were collected

Table 4-17. Overall *E. coli* source characterization and 95% confidence interval (CI) range for Segment 0805.

Segment 0805					
Category	**Source**	**Isolate #**	**Contribution (%)**	**95% CI Range**	
Avian	Avian	99	22.1	14.01	30.29
Avian	Waterfowl	17	3.8	0.05	7.55
Avian	**Subtotal**	**116**	**26.0**	**17.36**	**34.54**
Human	Sewage	0	0.0	0.00	0.00
Human	Wastewater	106	23.7	15.38	32.05
Human	**Subtotal**	**106**	**23.7**	**15.38**	**32.05**
Livestock	Bison	1	0.2	-0.70	1.15
Livestock	Bovine	29	6.5	1.66	11.32
Livestock	Donkey	3	0.7	-0.93	2.27
Livestock	Equine	1	0.2	-0.70	1.15
Livestock	Goat	3	0.7	-0.93	2.27
Livestock	Horse	12	2.7	-0.48	5.85
Livestock	**Subtotal**	**49**	**11.0**	**4.84**	**17.09**
Mammalian Wildlife	Armadillo	3	0.7	-0.93	2.27
Mammalian Wildlife	Coyote	0	0.0	0.00	0.00
Mammalian Wildlife	Deer	1	0.2	-0.70	1.15
Mammalian Wildlife	Opossum	2	0.4	-0.86	1.76
Mammalian Wildlife	Rabbit	1	0.2	-0.70	1.15
Mammalian Wildlife	Raccoon	27	6.0	1.37	10.71
Mammalian Wildlife	Rodent	29	6.5	1.66	11.32
Mammalian Wildlife	Skunk	0	0.0	0.00	0.00
Mammalian Wildlife	Squirrel	0	0.0	0.00	0.00
Mammalian Wildlife	**Subtotal**	**63**	**14.1**	**7.27**	**20.91**
Pet	Canine	6	1.3	-0.91	3.60
Pet	Cat	9	2.0	-0.74	4.77
Pet	Dog	36	8.1	2.72	13.39
Pet	Feline	0	0.0	0.00	0.00
Pet	**Subtotal**	**51**	**11.4**	**5.18**	**17.64**
Unknown	Unknown	62	13.9	7.10	20.64
Unknown	**Subtotal**	**62**	**13.9**	**7.10**	**20.64**
	Total	**447**	**100.0**		

Table 4-18. Runoff vs. non-runoff influenced *E. coli* source characterization for Trinity Segment 0805. (CI = confidence interval)

Segment 0805		Non-Runoff				Runoff			
Category	Source	Isolate #	Contribution (%)	95% CI Range		Isolate #	Contribution (%)	95% CI Range	
Avian	Avian	65	23.0	14.8	31.3	20	19.6	11.8	27.4
Avian	Waterfowl	6	2.1	-0.7	5.0	6	5.9	1.3	10.5
Avian	**Subtotal**	**71**	**25.2**	**16.7**	**33.7**	**26**	**25.5**	**16.9**	**34.0**
Human	Sewage	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Human	Wastewater	68	24.1	15.7	32.5	21	20.6	12.7	28.5
Human	**Subtotal**	**68**	**24.1**	**15.7**	**32.5**	**21**	**20.6**	**12.7**	**28.5**
Livestock	Bison	1	0.4	-0.8	1.5	0	0.0	0.0	0.0
Livestock	Bovine	17	6.0	1.4	10.7	8	7.8	2.6	13.1
Livestock	Donkey	3	1.1	-0.9	3.1	0	0.0	0.0	0.0
Livestock	Equine	1	0.4	-0.8	1.5	0	0.0	0.0	0.0
Livestock	Goat	3	1.1	-0.9	3.1	0	0.0	0.0	0.0
Livestock	Horse	6	2.1	-0.7	5.0	3	2.9	-0.4	6.3
Livestock	**Subtotal**	**31**	**11.0**	**4.9**	**17.1**	**11**	**10.8**	**4.7**	**16.9**
Mammalian Wildlife	Armadillo	2	0.7	-0.9	2.4	1	1.0	-1.0	2.9
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	1	0.4	-0.8	1.5	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	2	0.7	-0.9	2.4	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0	1	1.0	-1.0	2.9
Mammalian Wildlife	Raccoon	23	8.2	2.8	13.5	3	2.9	-0.4	6.3
Mammalian Wildlife	Rodent	16	5.7	1.1	10.2	6	5.9	1.3	10.5
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**44**	**15.6**	**8.5**	**22.7**	**11**	**10.8**	**4.7**	**16.9**
Pet	Canine	4	1.4	-0.9	3.7	2	2.0	-0.8	4.7
Pet	Cat	5	1.8	-0.8	4.4	4	3.9	0.1	7.7
Pet	Dog	21	7.4	2.3	12.6	10	9.8	4.0	15.6
Pet	Feline	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Pet	**Subtotal**	**30**	**10.6**	**4.6**	**16.7**	**16**	**15.7**	**8.6**	**22.8**
Unknown	Unknown	38	13.5	6.8	20.2	17	16.7	9.4	24.0
Unknown	**Subtotal**	**38**	**13.5**	**6.8**	**20.2**	**17**	**16.7**	**9.4**	**24.0**
	Total	**282**	**100**			**102**	**100**		

during events that could not be placed, definitively, in either the non-runoff or runoff categories; therefore, those isolates were excluded from this particular analysis as explained earlier in the introductory material for the results section.

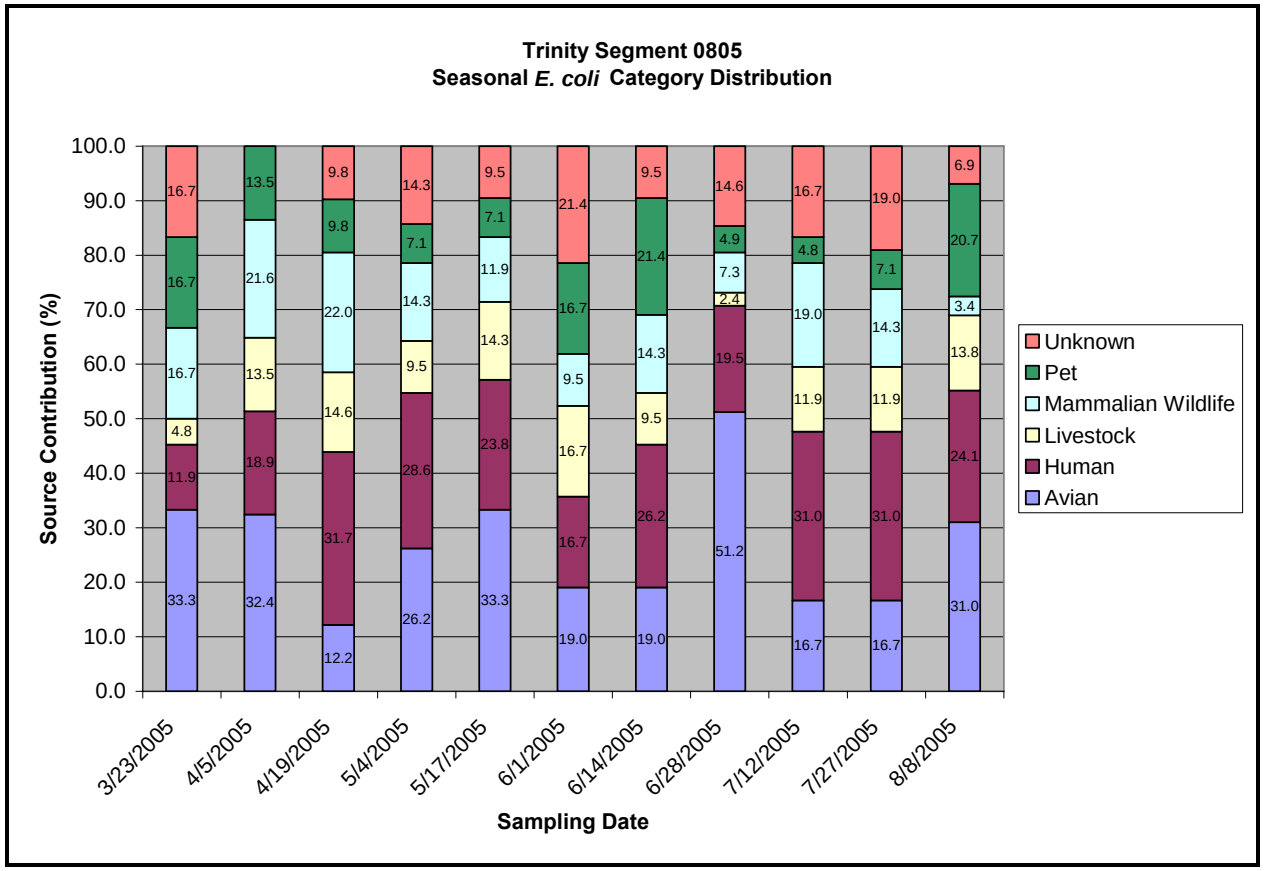
Avian, pet and unknown contributions were higher for samples containing >394 cfu/ 100ml than samples containing ≤394 cfu/ 100ml, and human, livestock and mammalian wildlife were higher for samples containing ≤394 cfu/ 100ml (Table 4-19). None of these differences, however, were statistically significant at α=0.05. Segment 0805 had a total of 350 *E. coli* isolates from samples with ≤394 cfu/ 100ml and 97 isolates from samples with >394 cfu/ 100ml, for a total of 447 isolates.

Table 4-19. *E. coli* source characterization for samples containing ≤394 cfu/100ml vs. those with >394 cfu/100ml for Segment 0841. (CI = confidence interval)

Segment 0805		Samples ≤ 394 cfu / 100ml				Samples > 394 cfu / 100ml			
Category	Source	Isolate #	Contribution (%)	95% CI Range		Isolate #	Contribution (%)	95% CI Range	
Avian	Avian	71	20.3	12.4	28.2	28	28.9	20.0	37.7
Avian	Waterfowl	12	3.4	-0.1	7.0	5	5.2	0.8	9.5
Avian	**Subtotal**	**83**	**23.7**	**15.4**	**32.1**	**33**	**34.0**	**24.7**	**43.3**
Human	Sewage	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Human	Wastewater	89	25.4	16.9	34.0	17	17.5	10.1	25.0
Human	**Subtotal**	**89**	**25.4**	**16.9**	**34.0**	**17**	**17.5**	**10.1**	**25.0**
Livestock	Bison	1	0.3	-0.8	1.3	0	0.0	0.0	0.0
Livestock	Bovine	26	7.4	2.3	12.6	3	3.1	-0.3	6.5
Livestock	Donkey	3	0.9	-0.9	2.7	0	0.0	0.0	0.0
Livestock	Equine	1	0.3	-0.8	1.3	0	0.0	0.0	0.0
Livestock	Goat	3	0.9	-0.9	2.7	0	0.0	0.0	0.0
Livestock	Horse	9	2.6	-0.5	5.7	3	3.1	-0.3	6.5
Livestock	**Subtotal**	**43**	**12.3**	**5.9**	**18.7**	**6**	**6.2**	**1.5**	**10.9**
Mammalian Wildlife	Armadillo	2	0.6	-0.9	2.0	1	1.0	-0.9	3.0
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	1	0.3	-0.8	1.3	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	2	0.6	-0.9	2.0	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0	1	1.0	-0.9	3.0
Mammalian Wildlife	Raccoon	24	6.9	1.9	11.8	3	3.1	-0.3	6.5
Mammalian Wildlife	Rodent	23	6.6	1.7	11.4	6	6.2	1.5	10.9
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**52**	**14.9**	**7.9**	**21.8**	**11**	**11.3**	**5.1**	**17.6**
Pet	Canine	5	1.4	-0.9	3.8	1	1.0	-0.9	3.0
Pet	Cat	5	1.4	-0.9	3.8	4	4.1	0.2	8.0
Pet	Dog	27	7.7	2.5	12.9	9	9.3	3.6	15.0
Pet	Feline	0	0.0	0.0	0.0	0	0.0	0.0	0.0
Pet	**Subtotal**	**37**	**10.6**	**4.5**	**16.6**	**14**	**14.4**	**7.5**	**21.3**
Unknown	Unknown	46	13.1	6.5	19.8	16	16.5	9.2	23.8
Unknown	**Subtotal**	**46**	**13.1**	**6.5**	**19.8**	**16**	**16.5**	**9.2**	**23.8**
	Total	**350**	**100.0**			**97**	**100.0**		

Temporal contributions of *E. coli* isolates by the various categories were analyzed and graphed in order to identify differences between sampling events and possibly determine whether or not there were temporal patterns present (Figure 4-12). The categorical contributions for Segment 0805 did not exhibit any strong seasonal patterns, and variations appear to be more random than systematically associated with time.

Figure 4-12. Segment 0805 temporal *E. coli* category distribution.



4.2.3.1 Station 10937- Mockingbird Lane (Dallas County)

Station 10937 results were based on 116 isolates. The largest contributor of *E. coli* contamination at station 10937 was from the human category, representing 28.4% of the total contribution (Figure 4-13; Table 4-20). Avian species contributed 25.0%, followed by mammalian wildlife with 18.1%. Unknown and pet categories were found to be the lowest contributors at this station 10937. Livestock were not represented at this particular station. When compared to the overall segment distribution, the results for station 10937 were substantially lower for the livestock category, which failed to be represented. The result is a 10.7% decrease from the average segment livestock contribution. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

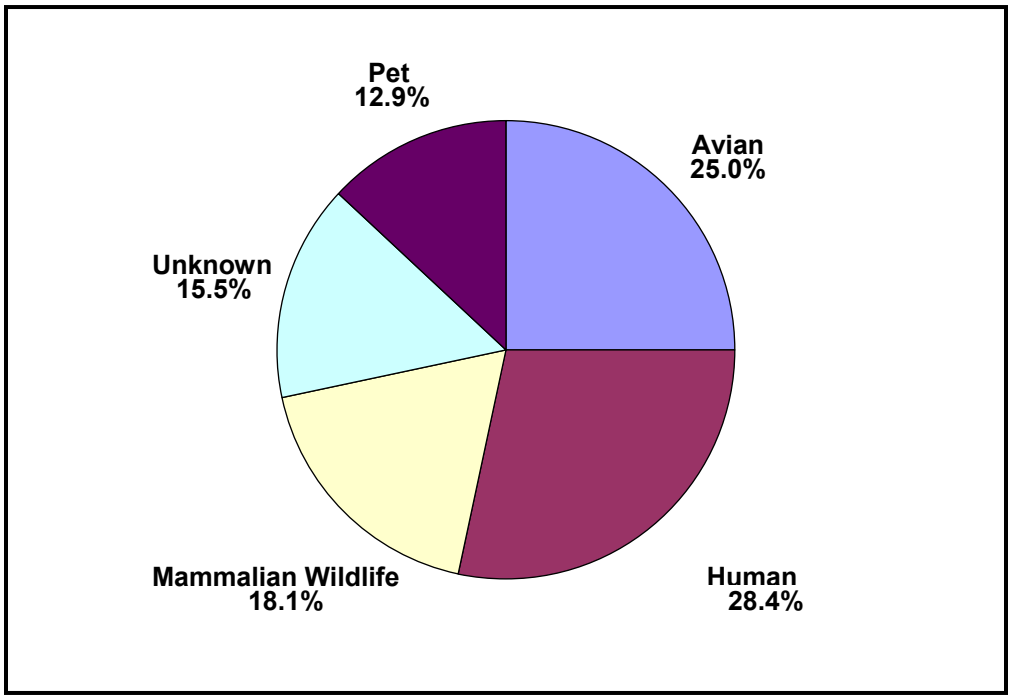


Figure 4-13. *E. coli* category characterization for station 10937 under all conditions.

Table 4-20. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 10937.

Station 10937					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	24	20.7	12.8	28.6
Avian	Waterfowl	5	4.3	0.3	8.3
Avian	**Subtotal**	**29**	**25.0**	**16.5**	**33.5**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	33	28.4	19.6	37.3
Human	**Subtotal**	**33**	**28.4**	**19.6**	**37.3**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	0	0.0	0.0	0.0
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	0	0.0	0.0	0.0
Livestock	**Subtotal**	**0**	**0.0**	**0.0**	**0.0**
Mammalian Wildlife	Armadillo	1	0.9	-0.9	2.7
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	8	6.9	1.9	11.9
Mammalian Wildlife	Rodent	12	10.3	4.4	16.3
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**21**	**18.1**	**10.6**	**25.7**
Pet	Canine	1	0.9	-0.9	2.7
Pet	Cat	1	0.9	-0.9	2.7
Pet	Dog	13	11.2	5.0	17.4
Pet	Feline	0	0.0	0.0	0.0
Pet	**Subtotal**	**15**	**12.9**	**6.4**	**19.5**
Unknown	Unknown	18	15.5	8.4	22.6
Unknown	**Subtotal**	**18**	**15.5**	**8.4**	**22.6**
	Total	**116**	**100.0**		

4.2.3.2 Station 10934- South Loop 12 (Dallas County)

Station 10934 results were based on 116 isolates. Thirty-one percent contribution of avian *E. coli* represented the highest percentage at station 10934 (Figure 4-14; Table 4-21). The next highest percentage was from the human category with 21.6%, while the unknown category was the third highest contributor with an average of 18.1%. Pet, mammalian wildlife, and livestock populations contributed the least.

Compared with the overall segment, the avian contribution was slightly higher at station 10934, 31.0% compared to the overall segment average of 26.0%. Also, the livestock contribution was 5.5% lower than the overall segment average. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

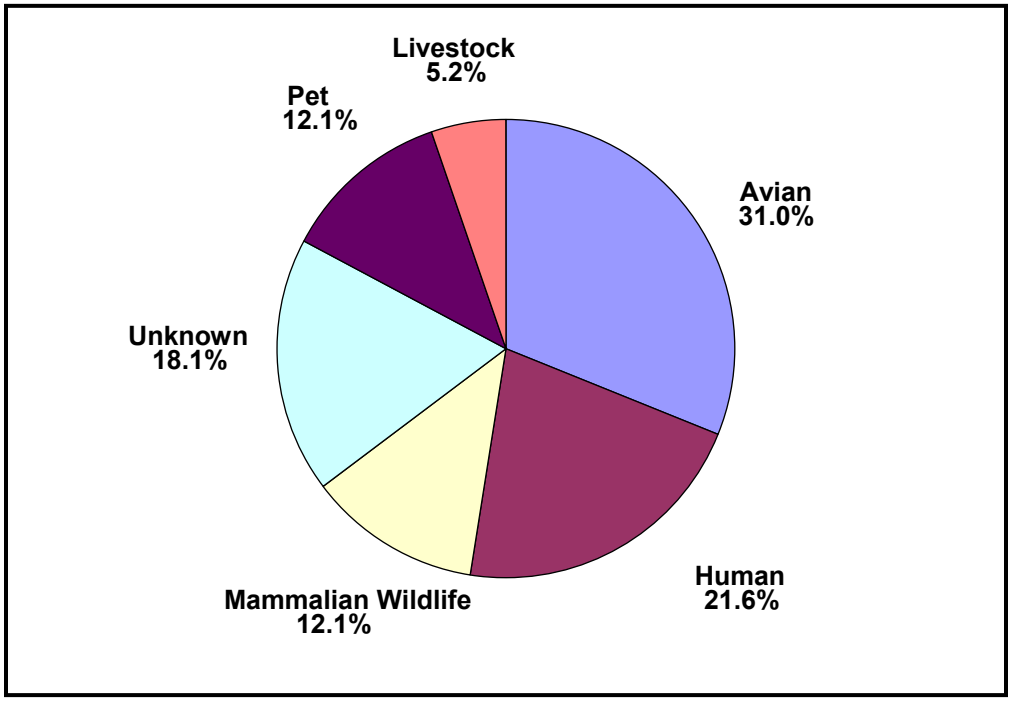


Figure 4-14. *E. coli* category characterization for station 10934 under all conditions.

Table 4-21. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 10934.

Station 10934					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	30	25.9	17.3	34.4
Avian	Waterfowl	6	5.2	0.8	9.5
Avian	**Subtotal**	**36**	**31.0**	**22.0**	**40.1**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	25	21.6	13.5	29.6
Human	**Subtotal**	**25**	**21.6**	**13.5**	**29.6**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	2	1.7	-0.8	4.3
Livestock	Donkey	0	0.0	0.0	0.0
Livestock	Equine	0	0.0	0.0	0.0
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	4	3.4	-0.1	7.0
Livestock	**Subtotal**	**6**	**5.2**	**0.8**	**9.5**
Mammalian Wildlife	Armadillo	1	0.9	-0.9	2.7
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	0	0.0	0.0	0.0
Mammalian Wildlife	Rabbit	1	0.9	-0.9	2.7
Mammalian Wildlife	Raccoon	5	4.3	0.3	8.3
Mammalian Wildlife	Rodent	7	6.0	1.4	10.7
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**14**	**12.1**	**5.7**	**18.5**
Pet	Canine	2	1.7	-0.8	4.3
Pet	Cat	4	3.4	-0.1	7.0
Pet	Dog	8	6.9	1.9	11.9
Pet	Feline	0	0.0	0.0	0.0
Pet	**Subtotal**	**14**	**12.1**	**5.7**	**18.5**
Unknown	Unknown	21	18.1	10.6	25.7
Unknown	**Subtotal**	**21**	**18.1**	**10.6**	**25.7**
	Total	**116**	**100.0**		

4.2.3.3 Station 10925- SH 34 (Ellis County)

Station 10925 results were based on 103 isolates. The *E. coli* population at station 10925 was led by an avian contribution of 24.3% (Figure 4-15; Table 4-22). The human category contribution represented 22.3% of the *E. coli* input, and the livestock contribution averaged 18.4%. Mammalian wildlife, unknown category, and pet population, respectively, were found to be the lowest *E. coli* contributors at station 10925. When compared with the overall segment, the largest difference was found to be associated with the livestock contribution, which was 7.7% greater at station 10925 than the overall segment average. This increase is probably a result of the largely rural area surrounding this station, resulting in an increased number of livestock present in the immediate watershed. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

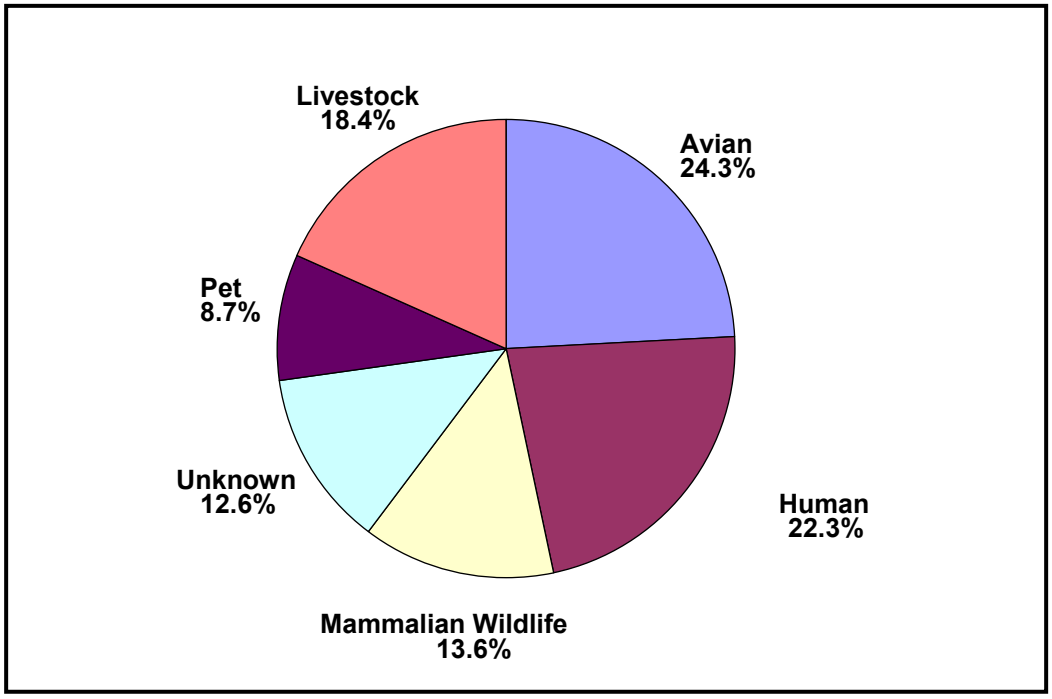


Figure 4-15. *E. coli* source characterization for station 10925 under all conditions.

Table 4-22. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 10925.

Station 10925					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	22	21.4	13.3	29.4
Avian	Waterfowl	3	2.9	-0.4	6.2
Avian	**Subtotal**	**25**	**24.3**	**15.9**	**32.7**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	23	22.3	14.2	30.5
Human	**Subtotal**	**23**	**22.3**	**14.2**	**30.5**
Livestock	Bison	1	1.0	-1.0	2.9
Livestock	Bovine	14	13.6	6.9	20.3
Livestock	Donkey	2	1.9	-0.8	4.6
Livestock	Equine	1	1.0	-1.0	2.9
Livestock	Goat	0	0.0	0.0	0.0
Livestock	Horse	2	1.9	-0.8	4.6
Livestock	**Subtotal**	**20**	**19.4**	**11.7**	**27.2**
Mammalian Wildlife	Armadillo	1	1.0	-1.0	2.9
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	0	0.0	0.0	0.0
Mammalian Wildlife	Opossum	1	1.0	-1.0	2.9
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	7	6.8	1.9	11.7
Mammalian Wildlife	Rodent	4	3.9	0.1	7.7
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**13**	**12.6**	**6.1**	**19.1**
Pet	Canine	0	0.0	0.0	0.0
Pet	Cat	1	1.0	-1.0	2.9
Pet	Dog	8	7.8	2.5	13.0
Pet	Feline	0	0.0	0.0	0.0
Pet	**Subtotal**	**9**	**8.7**	**3.2**	**14.3**
Unknown	Unknown	13	12.6	6.1	19.1
Unknown	**Subtotal**	**13**	**12.6**	**6.1**	**19.1**
	Total	**103**	**100.0**		

4.2.3.4 Station 10924- FM 85 (Navarro County)

Station 10924 results were based on 112 isolates. Avian population led the contribution of *E. coli* at station 10924, with 23.2% (Figure 4-16; Table 4-23). Human addition was second highest with 22.3%. Livestock were once again very high with 20.5% of the total contribution, while the mammalian wildlife, pet, and unknown categories contributed the least to the overall *E. coli* concentration.

When compared with the overall segment, the livestock and unknown categories showed the highest percent difference. The livestock contribution was found to be 9.8% higher for station 10924 than for the combined segment, and unknowns were lower. This station, like 10925, is largely rural in nature and has a higher density of livestock than station 10937 and 10934. The limited number of isolates at any one station precluded performance of any statistically meaningful analysis that involved further separation of the data, such as was performed on the aggregated data for the segment.

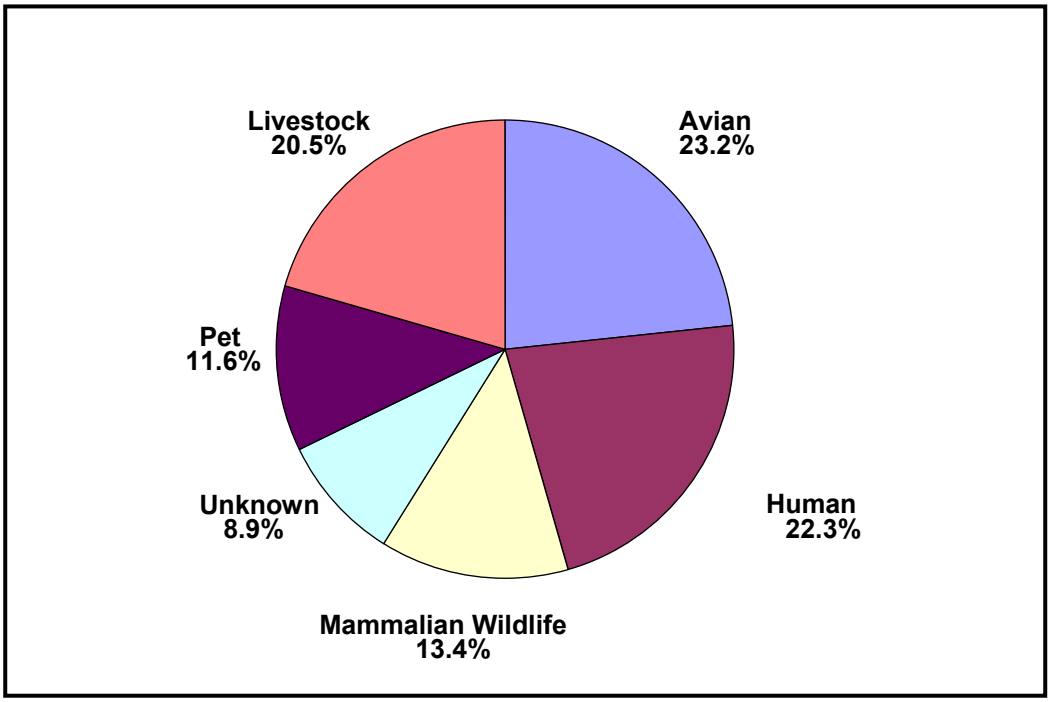


Figure 4-16. *E. coli* source characterization for station 10924 under all conditions.

Table 4-23. Overall *E. coli* source characterization and 95% confidence interval (CI) range for station 10924.

Station 10924					
Category	**Source**	**Isolate #**	**% Contribution**	**95% CI Range**	
Avian	Avian	23	20.5	12.6	28.5
Avian	Waterfowl	3	2.7	-0.5	5.8
Avian	**Subtotal**	**26**	**23.2**	**14.9**	**31.5**
Human	Sewage	0	0.0	0.0	0.0
Human	Wastewater	25	22.3	14.2	30.5
Human	**Subtotal**	**25**	**22.3**	**14.2**	**30.5**
Livestock	Bison	0	0.0	0.0	0.0
Livestock	Bovine	13	11.6	5.3	17.9
Livestock	Donkey	1	0.9	-1.0	2.7
Livestock	Equine	0	0.0	0.0	0.0
Livestock	Goat	3	2.7	-0.5	5.8
Livestock	Horse	6	5.4	0.9	9.8
Livestock	**Subtotal**	**23**	**20.5**	**12.6**	**28.5**
Mammalian Wildlife	Armadillo	0	0.0	0.0	0.0
Mammalian Wildlife	Coyote	0	0.0	0.0	0.0
Mammalian Wildlife	Deer	1	0.9	-1.0	2.7
Mammalian Wildlife	Opossum	1	0.9	-1.0	2.7
Mammalian Wildlife	Rabbit	0	0.0	0.0	0.0
Mammalian Wildlife	Raccoon	7	6.3	1.5	11.0
Mammalian Wildlife	Rodent	6	5.4	0.9	9.8
Mammalian Wildlife	Skunk	0	0.0	0.0	0.0
Mammalian Wildlife	Squirrel	0	0.0	0.0	0.0
Mammalian Wildlife	**Subtotal**	**15**	**13.4**	**6.7**	**20.1**
Pet	Canine	3	2.7	-0.5	5.8
Pet	Cat	3	2.7	-0.5	5.8
Pet	Dog	7	6.3	1.5	11.0
Pet	Feline	0	0.0	0.0	0.0
Pet	**Subtotal**	**13**	**11.6**	**5.3**	**17.9**
Unknown	Unknown	10	8.9	3.3	14.5
Unknown	**Subtotal**	**10**	**8.9**	**3.3**	**14.5**
	Total	**112**	**100.0**		

Section 5
Summary and Conclusions

During this study water samples were collected from Segments 0806, 0841, and 0805 of the Trinity River in order to quantify the *E. coli* concentrations in the waterbodies, and primarily to isolate representative colonies from the samples and track their sources through BST. BST is a process in which bacteria may be linked to their source hosts through their DNA by molecular tools, in this study, ribotyping. The study was initiated by the Texas Commission for Environmental Quality (TCEQ) in order to determine the extent and sources of bacteria contamination.

Water samples were collected during 11 events, between 23 March 2005 and 16 August 2005, while the known source fecal and sewage ribotype library sample collection occurred over 12 sampling events during the same period. Overall, 550 water samples were collected from 10 different stations along the West Fork and the Trinity River. Approximately two bacterial colonies (isolates) were isolated from each water sample, for a total of 1,135 isolates.

Following unknown isolate and known source ribotyping, the results were identified, matched, assigned to the proper category. The isolates for each segment were analyzed based on their relative contribution, runoff vs. non-runoff influenced, whether they were isolated from samples containing ≤394 cfu/ 100ml or from samples containing >394 cfu/ 100ml, and based on temporal differences.

The *E. coli* sources were relatively diverse in each of the three segments, with no one category dominating any of the stations or segments (Tables 5-1, 5-2, and 5-3). Avian species were the highest contributors of *E. coli* in each of the three segments; with the highest concentration associated with the uppermost segment, 0806, and progressively falling in each of the next two segments. However, there was less than a 2% difference between the avian contribution in Segment 0806 and Segment 0805. Human contribution was relatively low in Segment 0806, while it was relatively high in Segments 0841 and 0805. The human contribution rose steadily from Segment 0806 to Segment 0805. Mammalian wildlife was found to be a relatively high contributor in Segment 0806, falling steadily through Segment 0841 into Segment 0805. The unknown sources contributed a relatively large percentage in Segment 0806, but dropped steadily from Segment 0806 to Segment 0805. The pet contribution was found to be somewhat low and stable, with less than a 2% change between Segments 0806 and 0805, even though it did show a consistent drop from the uppermost site to the lowermost site. Livestock were consistently low in the upper two segments, each with 3.8%; however, their contribution rose dramatically to 10.7% in Segment 0805 and to an average of about 19% at the two most downstream and rural stations. Overall, each of the source contributors showed a definite trend, whether positive or negative, as one moves downstream from Segment 0806, through Segment 0841, and into Segment 0805. The categories did show consistencies in source species. The avian category was consistently dominated by non-waterfowl species, while the livestock category's contribution was shared by bovine and horses. Mammalian wildlife was found to be high in rodent species and raccoons, while the pet category was found to be consistently led by dogs.

Table 5-1. Summary *E. coli* source characterization summary for stations associated with Trinity Segment 0806.

Source	18459	10938	16120	Segment 0806 Total
Avian (%)	25.0	26.7	32.5	28.0
Human (%)	13.8	12.1	7.9	11.3
Mammalian Wildlife (%)	23.3	18.1	27.2	22.8
Unknown (%)	19.8	26.7	15.8	20.8
Pet (%)	10.3	15.5	14.0	13.3
Livestock (%)	7.8	0.9	2.6	3.8
Total	100.0	100.0	100.0	100.0

Table 5-2. Summary *E. coli* source characterization summary for stations associated with Trinity Segment 0841.

Source	17669	11081	11089	Segment 0841 Total
Avian (%)	28.3	31.3	23.7	27.8
Human (%)	19.5	18.3	23.7	20.5
Mammalian Wildlife (%)	18.6	13.0	19.3	17.0
Unknown (%)	16.8	20.0	17.5	18.1
Pet (%)	12.4	11.3	14.9	12.9
Livestock (%)	4.4	6.1	0.9	3.8
Total	100.0	100.0	100.0	100.0

Table 5-3. Summary *E. coli* source characterization summary for stations associated with Trinity Segment 0805

Source	10937	10934	10925	10924	Segment 0805 Total
Avian (%)	25.0	31.0	24.3	23.2	26.0
Human (%)	28.4	21.6	22.3	22.3	23.7
Mammalian Wildlife (%)	18.1	12.1	12.6	13.4	14.1
Unknown (%)	15.5	18.1	12.6	8.9	13.9
Pet (%)	12.9	12.1	8.7	11.6	11.4
Livestock (%)	0.0	5.2	19.4	20.5	11.0
Total	100.0	100.0	100.0	100.0	100.0

The remaining analyses, runoff vs. non-runoff influence and isolates from samples with ≤394 cfu/ 100ml vs. isolates from samples with >394 cfu/ 100ml, showed no statistically significant differences in sources when evaluated by segment. In addition, normalization of the data showed no significant difference from non-normalized data. Also, there were no temporal patterns in source contributions evident in any of the three segments.

In conclusion, the *E. coli* sources that were encountered were found to be diverse and non-dominating. This will result in a need for diversification of control measures and collaboration by landowners, governments, citizens, and other stakeholders to come up with a variety of methods for addressing this distinct situation.

SECTION 6
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